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Growth, 1942, **6**, 1-6.

TOLERANCE OF LARVAE OF DROSOPHILA FOR NUCLEIC ACID COMPONENTS: ADENINE*

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Wellesley College and the Marine Experimental Station of the Lankenau Hospital Research Institute

Baumberger (1919) suggested that yeast nucleoprotein satisfied the protein requirements for normal growth of *Drosophila melanogaster*. Tatum (1939) tested yeast nucleic acid in a medium containing, in addition to other food classes, hydrolyzed casein and tryptophane as nitrogen sources. He found that normal growth did not occur unless three unknown yeast factors were present. Lafon (1939) attempted to grow Drosophila on a mixture of amino acids but with no nucleic acid derivatives present and was unsuccessful. A preliminary step to any attempt at preparation of a mixture of the components of protein, capable of maintaining normal growth in Drosophila larvae, is the determination of the tolerance of the larvae for each proposed compound. The purpose of this investigation was to determine the concentration of adenine sulphate which is toxic for the larvae of Drosophila. In addition it was aimed to determine the highest concentration in which normal growth, as indicated by larval length, could be maintained.

Culture methods for stock, egg collecting, and sterilization procedure were the same as described by Wilson (1941). Preliminary experiments, in which adenine sulphate was the only source of nitrogen aside from that present in the agar, were unsuccessful in producing growth and the larvae died within 72 hours. There was a suggestion, however, that M/500 and M/250 adenine sulphate in the experimental medium were toxic. It was decided, therefore, to test the various concentrations of adenine sulphate on the growth of larvae when they were fed on a concentration of dead yeast sufficient to produce pupation between 120 and 128 hours after oviposition.

Autoclaved culture medium prepared as described by Schadar

*Supported by a grant from The Women's Auxiliary of the Lankenau Hospital Research Institute for cancer research.

[1]I wish to thank Dr. Frederick S. Hammett and the Staff of the Lankenau Hospital Research Institute for criticism and aid in making this work possible, and Miss Barbara Croll of Wellesley College for technical assistance.

(1941) for her control experiments and supplemented by the addition of adenine sulphate in distilled water was used in the test. No adenine was added to the control medium. While hot, the media were poured into sterile 5 cm. Petrie dishes and allowed to harden before the addition of sterile eggs.

Two series of four experiments were performed. For each experiment of the first series, eggs from the same group of parents were added to control dishes and to tests containing respectively M/250, M/500, and M/1000 adenine. In the second series, eggs were added to control dishes and to tests containing M/2000, M/4000, and M/6000 adenine. Approximately 20 sterilized eggs obtained over a two-hour laying period were used in each culture dish. Tests and controls, run simultaneously, were kept in an incubator at $25\pm1°C$. Forty-eight hours after the mean hour of egg laying, three dishes of each type of medium were opened and the larvae killed and measured as described by Alpatov (1929). This method of immersion in hot water caused the larvae to lie straight with no stretching or curling and it was then possible to measure the body length with an ocular micrometer in a 10 X ocular and a 48 mm. objective. Other series of three dishes were opened and measured at 72, 96, and 120 hours after oviposition.

Glassware and media were kept sterile by standard bacteriological technique. Turbidity tests with bacto peptone glucose broth were made on each culture dish and all measurements of larvae from contaminated dishes were discarded.

Results

Tables 1 and 2 give the average length in millimeters for larvae killed at the stated hours. The averages include data from all four experiments in each series. These same data are presented graphically in Figures 1 and 2.

Concentrations below M/500 have no effect on the growth of first and second instar larvae. All concentrations above M/6000 retard the ecdysis from second to third instars and retard the rate of growth during the third stadium. This latter fact results in a postponement of puparium formation. In addition M/250 concentration is definitely toxic. Few larvae so treated reach the third stadium and all eventually die before puparium formation.

There is a lag in moulting for concentrations of M/500. Larvae of the first instar are found after 72 hours and a few animals remain

TABLE 1

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH ADENINE SULPHATE

Age in hours from median hour of oviposition

AGE	CONTROL			M/250			M/500			M/1000		
	Instar	No.	Length	Instar	No.	Length	Instar	No.	Length	Instar	No.	Length
48	1	86	1.63	1	166	1.46	1	112	1.60	1	114	1.60
	2	96	2.13	2	8	1.87	2	68	2.06	2	56	2.11
72				1	24	1.50	1	10	1.35	1	1	1.65
	2	99	2.93	2	172	2.31	2	112	2.71	2	108	3.05
	3	41	4.04				3	18	3.73	3	24	3.84
96				1	2	1.50						
	2	1	3.22	2	92	2.49	2	14	2.90	2	2	3.07
	3	144	5.05	3	12	3.32	3	98	4.68	3	110	4.84
120				1	2	2.02						
				2	18	2.26	2	16	2.51			
	3	40	4.88	3	36	3.91	3	112	5.19	3	160	4.50
		101 pupae			Dead larvae						4 pupae	
144		All pupated			All larvae dead			All pupated			All pupated	

TABLE 2

AVERAGE LENGTH IN MILLIMETERS OF LARVAE TREATED WITH ADENINE SULPHATE

Age in hours from median hour of oviposition

AGE	CONTROL			M/2000			M/4000			M/6000		
	Instar	No.	Length	Instar	No.	Length	Instar	No.	Length	Instar	No.	Length
48	1	50	1.59	1	106	1.65	1	52	1.72	1	43	1.60
	2	110	2.04	2	105	2.01	2	88	1.99	2	101	2.05
72	2	90	3.16	2	140	3.06	2	121	3.09	2	60	2.98
	3	130	3.72	3	60	3.61	3	19	3.51	3	98	3.79
96	3	120	4.96	3	154	4.33	3	168	4.40	3	141	4.92
120	3	202	5.10	3	121	5.23	3	146	5.27	3	162	5.20
144		All pupated			82% pupated			All pupated			All pupated	

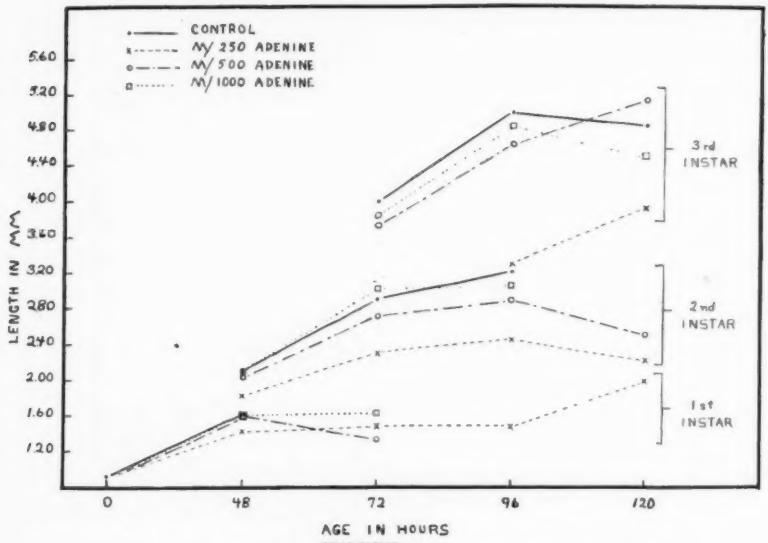

FIGURE 1

RATE OF GROWTH FOR LARVAE OF THE FIRST SERIES OF EXPERIMENTS

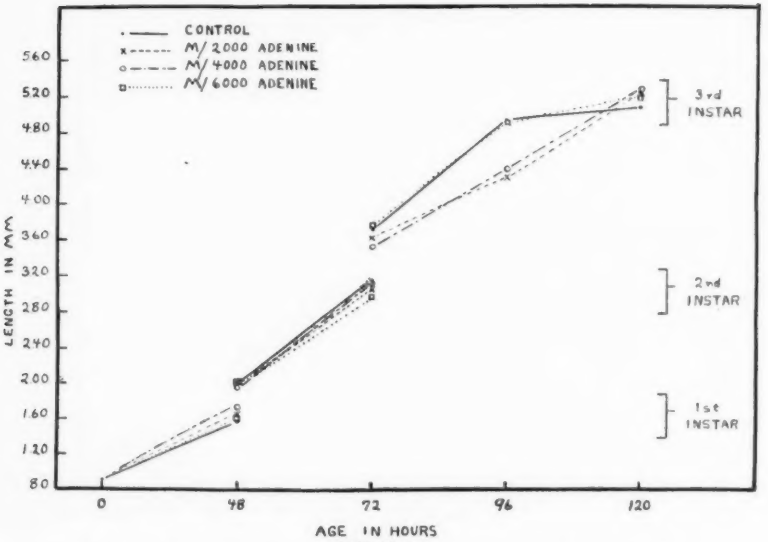

FIGURE 2

RATE OF GROWTH FOR LARVAE OF THE SECOND SERIES OF EXPERIMENTS

in the second instar even up to 120 hours. These eventually pupate, for no larvae remain at 140 hours and their number indicates that few, if any, deaths have occurred. Slow growth of larvae exposed to M/2000 and M/4000 results in later moulting from second to third stadia and in an increase of the time before puparium formation. It is thus noted in Table 2 that animals in the third instar at 72 hours are smaller than the controls but at 120 hours are larger than the controls. Shortening in preparation for puparium formation has already begun in the controls. This phenomenon of decrease in average length is also illustrated in Figure 1 by a comparison of the larvae at 120 hours when treated with M/500 adenine with those receiving no adenine. This marked shortening of the control indicates the imminence of puparium formation. On the other hand this process has not yet started in the treated animals.

That the adenine is not effective in retarding growth until after the second moult may indicate either that there is a cumulative effect or that some specific process concerned with length growth is retarded during the first stadium when larvae are treated with M/250 adenine. Viability of germ cells is not affected for fertile adults are produced in all concentrations above M/250. Proof of this is seen in subsequent emergence of adult offspring. Hammett (1938) found adenine toxic to Obelia below a concentration of M/6250. That a concentration within this same range marks the limits of toxicity for Drosophila larvae is evidence for the thesis that studies of this nature may have general applicability to animals.

Summary

Adenine sulphate in concentrations of M/250 is lethal to larvae of *Drosophila melanogaster*.

Growth in length and formation of puparia are definitely retarded by concentrations of M/500 adenine sulphate.

Growth in length is slightly retarded and formation of puparia delayed by concentrations between M/1000 and M/6000 adenine.

Normal growth and puparium formation occurs in Drosophila larvae treated with M/6000 adenine.

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Growth, 1942, **6**, 7-22.

THE EFFECTS OF CONTINUED ORAL ADMINISTRATION OF STILBESTROL ON BODY GROWTH AND ORGAN WEIGHTS OF ADULT UNCASTRATED AND CASTRATED FEMALE RATS

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The effects of prolonged administration of estrogenic substances on body growth and weights of organs not only of the normal female but also of the castrated female animal are of considerable interest since in the realms of human therapy these two types of animals represent two distinct clinical entities. Most of the work of this nature has been done on normal immature laboratory animals. A review of the literature further reveals that few writers have subjected their data to statistical analysis. Noteworthy exceptions are the papers by Freudenbergen and Clausen (1937*A* and *B*) and Clausen and Freudenbergen (1939) who studied the effects of large dosages (10, 20 and 36*r* daily) of theelin on immature normal female rats and analysed the resulting data by statistical methods. In view of these facts data collected in conjunction with a histological study (Russel, *et al.*, 1941) of the effects of the prolonged oral administration of stilbestrol on adult uncastrated and castrated female rats are presented in this paper.

A. MATERIALS AND METHODS

Normal and castrated albino female rats, of Wistar stock inbred for many generations were used in this experiment. The diet consisted of Purina Dog Chow and water. The castrated control and castrated treated animals were of a comparable chronic state of castration, oophorectomy having been performed approximately one month previously.

The crystallin stilbestrol used throughout was chemically pure. As stilbestrol is not soluble in water it was given either in 40 per cent ethyl alcohol or as the sodium salt dissolved in water. The alcoholic solution containing 250 gamma per cubic centimeter was made by simple

The authors wish to thank Burroughs Wellcome and Co. for material and aid furnished for this experiment.

solution. The solution of the salt was made by dissolving 20 mg. of stilbestrol in 0.5 cubic centimeters of *N* NaOH and adding sufficient distilled water to make solutions containing 250 gamma and 50 gamma per cubic centimeter. Due to the fact that the aqueous alkaline solutions turn brown on standing they were prepared fresh before each administration. One cubic centimeter of the medication was given three times a week by stomach tube without anesthesia (Matthews, *et al.*, 1941), for 90 days in the case of the uncastrated females and for 50 days in the case of the castrated females.

A total of 125 animals were used in this study. Twenty-five uncastrated virgin female rats received the alkaline aqueous solution containing 50 gamma per cc. and 12 castrated females likewise received this same dose. A group of 25 uncastrated virgin females and a group of 12 castrated females both received the alkaline aqueous solution containing 250 gamma per cc. Eleven uncastrated virgin females received the solution containing 250 gamma per cc. in 40 per cent ethyl alcohol. Four groups of controls were used. One group of fifteen uncastrated females received no treatment of any kind. Another group was composed of 15 untreated castrated females. In addition, to check the effects of the two solvents *per se*, one group of five uncastrated female rats was given the NaOH solution of the same normality as the alkaline aqueous solution of stilbestrol. A second group of five similar animals was treated with 40 per cent ethyl alcohol only.

Vaginal smears were taken daily by the pipette method (Emery and Schwabe, 1936) and body weights recorded every five days. The animals were killed by ether anesthesia, the organs carefully dissected free of adherent issue and weighed immediately. To ascertain if any real difference existed between the control and treated groups the resulting data were subjected to statistical analysis. When the difference between the two means divided by the standard error of the difference (subsequently referred to as the "significant ratio") gave a value of three or over, the difference between the two means was considered significant, while a figure below three was taken to indicate that no real change had occurred.

B. Results

1. *Growth and Body Weight Observations*

A casual inspection of the weight curves of our animals (Figure 1), will reveal that the sodium salt of stilbestrol has a profound influence on the growth of the adult rat. The average initial body weights of the

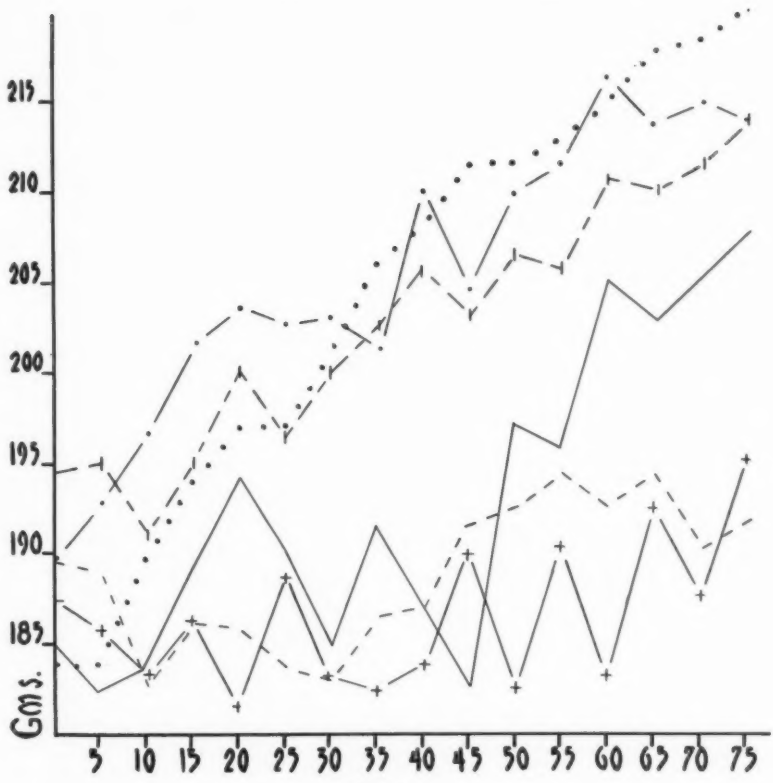

FIGURE 1

GROWTH CURVES OF UNCASTRATED TREATED AND CONTROL ADULT FEMALE RATS
— — — 25 rats treated three times weekly with 50 gamma of stilbestrol in aqueous alkaline solution. + ——— + 25 rats treated with 250 gamma of stilbestrol in aqueous alkaline solution. ——— 12 rats treated with 250 gamma stilbestrol in 40 per cent ethyl alcohol. . . . . 15 untreated controls. . —— . 5 controls treated with 40 per cent ethyl alcohol only. |— ——— —| 5 controls treated with the aqueous alkaline medium only. Abscissa in days.

uncastrated females receiving 50 gamma and 250 gamma of stilbestrol in alkaline aqueous solution were nearly identical being 189 and 187 grams, respectively; and these weights were also similar to the initial weight (184 gms.) of the control animals (Groups *A*, *B*, *C*, Table 1). The weights at the termination of treatment disclosed that the average body weight of the uncastrated 50 gamma group was 192 grams or

TABLE 1

BODY WEIGHT IN GRAMS AT THE BEGINNING AND END OF THE EXPERIMENT
Groups *A* and *B* treated with the sodium salt of stilbestrol for a period of 90 days.
Groups *D* and *E* same treatment for a period of 50 days.

$$\textit{Coefficient of Variation} = \frac{SD + 100}{\text{Mean}}$$

$$\textit{Standard error of Difference} = \sqrt{(SE_1)^2 + (SE_2)^2}$$

$$SE = \textit{Standard error of Mean} = \frac{SD}{\sqrt{N}}$$

$$SD = \textit{Standard Deviation} = \sqrt{\frac{\Sigma d^2 f}{n}}$$

Group and number		Mean Start	Mean Finish	Percent Deviation	Mean Difference / S E of Difference	Coefficient of Variation Start	Coefficient of Variation Finish	Range Start	Range Finish
	Uncastrated							217—	127—
A-25	50 Gamma	189	192	+1.5	0.6	9.7	6.4	162	170
	Uncastrated							209—	223—
B-25	250 Gamma	187	195	+4.8	2.0	7.7	8.0	155	165
	Normal							206—	238—
C-15	Controls	184	220	+19.5	6.8	6.5	6.6	162	195
	Castrated							235—	230—
D-12	50 Gamma	206	200	—3.0	0.9	8.3	9.2	176	172
	Castrated							234—	237—
E-12	250 Gamma	208	208	—	—	7.8	8.4	185	180
	Uncastrated							203—	225—
F-11	Alc. 250 Gamma	185	208	+12.4	3.0	7.2	8.8	165	178
	Castrated							234—	257—
G-15	Controls	195	225	+15.0	3.2	10.4	11.5	165	178

13 per cent less than that of the untreated controls, while the average weight of those which received 250 gamma was 195 grams or 11.4 per cent lower than the average of 220 grams obtained for the control animals (Table 1). The significance of these figures was further revealed by the "significant ratios" which were in these two groups 5.5 and 4.5 respectively. Table 1 also shows in juxtaposition the analysis of the mean body weight of each group of rats at the inception and termination of treatment. The control animals increased 19.5 per cent in weight during the period of the experiment, while the uncastrated female groups which received stilbestrol in NaOH solution gained only 1.5 per cent and 4.8 per cent when the treatment was 50 and 250 gamma respectively (Groups *A*, *B*, Table 1). The "significant ratio" for the control group is 6.8; for the 50 gamma group 0.6; and for the 250 gamma group 2.0 (Table 1). From these results it can be concluded that the control animals gained considerable over the

period of our experiment while the normal females treated with the sodium salt of stilbestrol experienced only insignificant increases in body weight (Groups *A, B, C,* Table 1).

The castrated females treated with 50 gamma of stilbestrol in NaOH solution sustained a loss in body weight of 3.0 per cent. This decrease in weight was not significant, however, as shown by a value of 0.9 for the "sigificant ratio" (Table 1). The castrated females treated with 250 gamma in NaOH solution had the same average weight of 208 gms. at the end of treatment as at the beginning of the experiment. The uncastrated females treated with 250 gamma of stilbestrol in 40 per cent ethyl alcohol gained 23 grams in weight or 12.4 per cent. The value of 3.0 for the "significant ratio" indicates that this group of animals experienced a gain in weight which was real (Table 1). (Compare Groups *D, E,* with *G,* Table 1.)

2. *Estrus Cycles*

The vaginal smears were taken daily and from these data the mean per cent of time in estrus for 90 days was obtained by the method described by Emery (1931) and found to be 73.3 per cent for the females treated with 50 gamma and 72.1 per cent for those given 250 gamma in alkaline aqueous solution. The females given 250 gamma in 40 per cent ethyl alcohol had a mean percentage of time in estrus of 62.0. Emery, *et al,* (1940), working with the same colony of rats have shown that the average per cent of time in estrus for a group of 75 untreated rats was 41.0 per cent. This compares favorably with approximately 37.0 per cent reported 10 years ago (Emery, 1931) 40.3 per cent in 1937 (Emery, 1937), and 38.7 per cent obtained in this study. Considering the fact that these dosages were very unphysiological it is interesting to note that we did not produce continuous estrus. Emery (1937) observed this same phenomenon in normal female rats using large doses of estrone. This also confirms the effect of theelol (as high as 65 gamma daily) in the case of normal female rats as reported by Wade and Doisy (1935). The castrated rats treated with stilbestrol (Groups *D and E,* Table 1) showed no cyclic variation and cornified squamous epithelium was always present in the smears. This effect was not obtained by Wade and Doisy (1935), a rhythmic response being maintained by their castrated animals. Nelson (1935) observed continuous estrus in castrated rats treated with 40 R.U. of estrone daily. Reece (1938) injected 100 I. U. of Progynon *B* into oophorectomized rats and reported that cornified cells were always present. Mark and Biskind (1941) also noted persistent estrus in castrated

female rats implanted with pellets of estrone for 32-237 days. Since our castrated animals were always in estrus and showed no daily variation they were not included in Figure 2.[1]

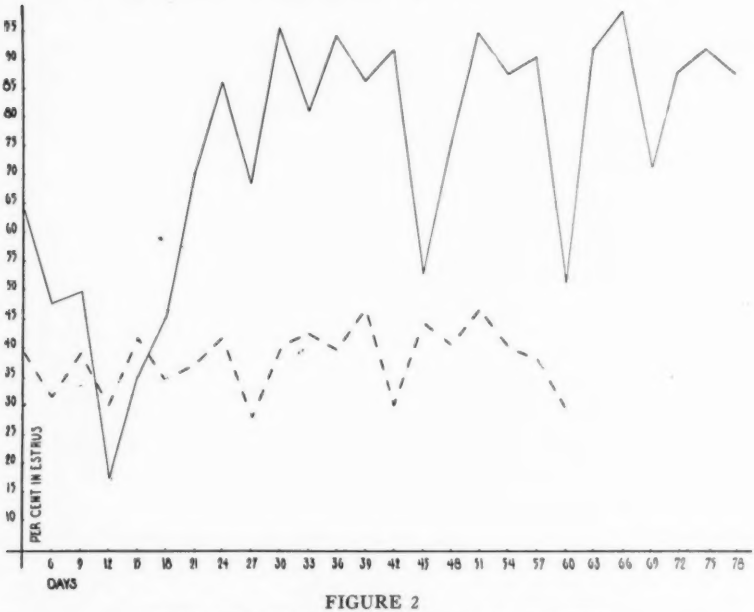

FIGURE 2

CURVES REPRESENTING PERCENTAGE OF RATS IN ESTRUS

Solid line 25 uncastrated females treated three times weekly with 250 gamma of stilbestrol in aqueous alkaline solution. Dashed line represents the untreated controls of Group *C*, Table 1.

3. *Weight of Individual Organs*

Since a preliminary analysis revealed that no differences existed between the uncastrated female groups which received the 50 gamma and 250 gamma of stilbestrol in NaOH solutions these groups are combined for subsequent discussion and tabulation (Table 2). Since the castrated groups which received the 50 gamma and 250 gamma in NaOH solutions were treated likewise and found to be similar it was thought best to combine these two dosages and then analyse the organ weights (Table 3).

a. Pituitary. That pituitary enlargement follows excessive estrogen treatment is a commonly observed phenomenon. Grumbrecht (1940)

[1]See footnote at end of paper.

TABLE 2

ORGAN WEIGHTS IN MGM. PER 100 GRAMS BODY WEIGHT OF 15 CONTROLS (GROUP *C*, TABLE 1) AND 50 TREATED RATS (GROUPS *A* AND *B* COMBINED, TABLE 1)

$$\textit{Coefficient of Variation} = \frac{SD + 100}{\text{Mean}}$$

$$\textit{Standard error of Difference} = \sqrt{(SE_1)^2 + (SE_2)^2}$$

$$SE = \textit{Standard error of Mean} = \frac{SD}{\sqrt{N}}$$

$$SD = \textit{Standard Deviation} = \sqrt{\frac{\Sigma d^2 f}{n}}$$

Uncastrated females	Mean weight Controls	Mean weight Treated	Percent change	Mean Diff. / S E of Diff.	Coeff. of Variation Controls	Coeff. of Variation Treated	Range Controls	Range Treated
							8.0	9.6
Pituitary	5.1	6.8	+33.3	4.9	21.5	19.0	3.5	4.4
							33.1	33.5
Adrenals	28.7	24.9	—13.6	1.4	11.9	56.0	22.6	18.9
							50.4	13.1
Ovaries	30.3	7.2	—76.9	9.6	25.0	96.6	21.9	3.0
							8.1	12.0
Thyroids	5.8	7.3	+32.7	2.2	16.3	28.0	4.3	5.7
							245	128
Thymus	185	105	—43.3	7.9	18.3	15.1	112	75
							420	395
Uterus	204	283	+38.7	4.4	43.9	18.0	103	191
							4562	5101
Liver	4198	3958	— 5.8	1.4	6.8	14.6	3657	2903
							887	908
Kidneys	749	793	+ 5.7	1.9	9.5	6.8	706	634
							768	651
Spleen	394	370	— 6.1	0.4	46.3	34.3	183	203

TABLE 3

ORGAN WEIGHTS IN MGM. PER 100 GRAMS BODY WEIGHT OF 15 CONTROLS (GROUP *G*, TABLE 1) AND 24 TREATED RATS (GROUP *D* AND *E* COMBINED, TABLE 1)

$$\textit{Coefficient of Variation} = \frac{SD + 100}{\text{Mean}}$$

$$\textit{Standard error of Difference} = \sqrt{(SE_1)^2 + (SE_2)^2}$$

$$SE = \textit{Standard error of Mean} = \frac{SD}{\sqrt{N}}$$

$$SD = \textit{Standard Deviation} = \sqrt{\frac{\Sigma d^2 f}{n}}$$

Castrated females	Mean weight Controls	Mean weight Treated	Percent change	Mean Diff. / S E of Diff.	Coeff. of Variation Controls	Coeff. of Variation Treated	Range Controls	Range Treated
							6.4	7.7
Pituitary	4.9	6.7	+36.7	6.6	18.4	8.7	3.1	5.7
							33.0	29.7
Adrenals	20.4	21.2	+ 3.9	0.5	30.8	12.8	13.2	19.1
							8.3	8.1
Thyroids	5.4	6.9	+27.7	3.3	2.2	14.1	3.8	5.4
							404	170
Thymus	187	122	—34.8	5.4	17.4	23.2	70	71
							168	230
Uterus	67	174	+159.6	9.4	54.4	14.8	30	125
							4055	5605
Liver	3374	4260	+26.2	6.4	14.3	5.1	2615	3641
							774	809
Kidneys	660	740	+12.1	4.3	8.0	7.1	576	630
							773	358
Spleen	438	254	—42.1	4.8	33.2	15.0	267	204

treated rats daily with 0.1 mg. estradiol monobenzoate and found a progressive increase in the pituitary weight up to 200 days. Immature female rates receiving 200 I.U.of theelin in oil daily showed a significant increase in the weight of the pituitary (Freudenberger and Clausen, 1937 *B*; and Clausen and Freudenberger, 1939). Bacsich and Folley (1939) found that the hypophysis of oophorectomized rats (20 daily injections of 1 mg. estradiol monobenzoate) was on the average almost three times heavier than in the case of ovariectomized controls. Nobel (1939) investigated the effect of stilbestrol on the pituitary of normal female adult rats and observed an increase in the size of this gland. In our experiments all the rats treated with stilbestrol showed a significant increase in weight of the pituitary and an analysis of the control and stilbestrol treated rats revealed a "significance ratio" of 4.9 for the uncastrated female series and 6.6 for the castrated female series (Tables 2 and 3).

b. Adrenals. Conflicting results have been reported concerning the effect of estrogens on the adrenal glands. Bacsich and Folley (1939) found no statistically significant differences between their treated ovariectomized rats and the control group. Freudenberger and Clausen (1937*A* and *B*) reported no significant change in the weight of the adrenals of immature animals treated with theelin, thus confirming the earlier work of Leonard, Meyer, and Hisaw (1931). Grumbrecht (1940) reports that animals treated 1.5 years with 0.1 mg. estradiol monobenzoate daily showed a normal adrenal weight range. Leiby (1933) reported adrenal enlargement in ovariectomized female rats after treatment with 2 mg. of theelol daily for six days. Mark and Biskind (1941) reported definite adrenal enlargement in the case of normal and castrated female rats implanted with estrone pellets for 27-237 days. Zondek (1936) and Clausen and Freudenberger (1939) showed that the adrenals were smaller in females treated with estrin. Nobel (1939), working with stilbestrol, stated that large dosages of this drug, given orally for 28 day to adult female rats produced enlargement of the adrenals. Mellish, Baer, and Macias (1940) found no change of adrenal weight in immature female rats implanted with 10 mg. of stilbestrol for 26 days. In both our normal and castrated groups no real change in adrenal weight developed as indicated by the "sigificant ratios" of 1.4 and 0.5 respectively (Tables 2 and 3). It is interesting to note that although the castrated rats were heavier than the uncastrated (Table 1) their adrenals were smaller (Tables 2, 3), thus confirming previous reports that castra-

tion of female rats reduces adrenal weight (Andersen and Kennedy, 1932; Winter and Emery, 1936).

c. Ovaries. A decrease of 76 per cent in the weight of the ovaries of our adult rats treated with stilbestrol resulted in a "significant ratio" of 9.6 (Table 2). Variation in the response of different animals to treatment is well exemplified by the value of the coefficient of variation, 96.6; whereas in the untreated females it was 25.0 (Table 2). According to Leonard, Meyer, and Hisaw (1931), and Mazer, Israel, and Alpus (1936), a decrease in the weight of the ovaries is the usual response of animals treated for prolonged periods with estrogenic substances.

d. Thyroids. To our knowledge no data have been published showing the effects of stilbestrol on the weight of the thyroid gland of the rat. Bacsich and Folley (1939) reported that the thyroid glands of both uncastrated and ovariectomized rats treated with estradiol monobenzoate weighed less than those of their control groups; however in the castrated and normal groups this was not statistically significant. Freudenberger and Clausen (1937*A*) found a significant decrease in thyroid weight of their theelin treated rats. This is in contradiction to the report of Reece (1938) who found that in a group of 10 ovariectomized rats injected with Progynon-*B* each thyroid was heavier than its control. Leiby (1933) also found that this gland increased in weight in the case of ovariectomized rats after estrogen treatment. In our own work the mean weight of the thyroid glands of the uncastrated rats treated with stilbestrol was 32.7 per cent more than that of the uncastrated control group. However, due to the variability of the data as reflected in a coefficient of variation of 28.0, the "significant ratio" is only 2.2 (Table 2). A similar comparison of the treated and control castrated groups gives a "significant ratio" of 3.3 (Table 3). Since the treated castrated animals showed an average increase in thyroid weight of 27.7 per cent which is somewhat smaller than the increase of 32.7 per cent obtained in the uncastrated group, the significant change in the weight of this latter group was due to the data being more closely arranged about the mean and this reflects itself in a small coefficient of variation (14.1, Table 3) as compared to that obtained in the uncastrated treated group (28.0, Table 2).

e. Thymus. It is the general consensus of opinion that the thymus decreases in weight when animals are treated with estrogenic substances (Golding and Ramirez, 1928; Zondek, 1936; Jacobsen, 1938).

From Tables 2 and 3 it will be seen that the decrease in thymus weight for the uncastrated females amounted to 43.3 per cent and for the castrated females 34.8 per cent. The "significant ratio" is 7.9 and 5.4 respectively for these two groups (Tables 2 and 3). Thus these data obtained with stilbestrol confirm existing reports on the effects of other estrogenic substances on the thymus gland.

f. Uterus. An inspection of Tables 2 and 3 will reveal that the uterus is markedly enlarged by stilbestrol. In the case of our treated female rats the per cent increase in weight and the "significant ratios" for the uncastrated and castrated groups are 38.7 per cent and 159.6 per cent and 4.4 and 9.4 respectively (Tables 2, 3). Freudenberger and Clausen (1937*A*) point out that the coefficient of variation for their control animals was markedly larger than for the treated groups. This phenomenon is confirmed in both our uncastrated and castrated control groups; the values for the coefficient of variation being 43.9 and 54.4, and 18.0 and 14.8 for the uncastrated and castrated control group and treated groups respectively (Tables 2 and 3). Spencer, D'Amour, and Gustavson (1932) report that the uteri weighed only 53 per cent as much in their estrin-injected rats (20 R.U. daily) as those of the controls. Their data therefore differs widely from that obtained in this study.

g. Liver. Selye (1939) reports that the total weight of the liver may increase considerably under the influence of chronic treatment with estradiol or stilbestrol. Other workers, Freudenberger and Clausen (1937*A*), and Korenchevsky and Dennison (1934), report that estrogens induced only insignificant decreases in liver weight. While our uncastrated females experienced a decrease of 5.8 per cent in mean liver weight, the analysis would indicate that this may not be due to the action of the drug since the "significant ratio" is only 1.4 (Table 2). A 26.2 per cent increase in the weight of the liver occurred in the case of our castrated rats treated with stilbestrol and the "significant ratio" is 6.4 (Table 3). Thus it would seem from the above citations and our own data that the precise effect of stilbestrol on the weight of the liver is obscure.

h. Kidneys. Korenchevsky, Burbank, and Hall (1939), report that immature ovariectomized rats receiving the dipropionate and benzoate butyrate esters of estradiol, showed a pronounced enlargement of the kidneys. Freudenberger and Clausen (1937*A*) observed a significant loss of weight in the case of their normal immature rats treated with estrin. In this study the uncastrated group which received

stilbestrol showed only a slight 5.7 per cent increase in kidney weight over that of the untreated animal (Table 2). On the other hand the mean value of the kidney weights of the castrated group which received stilbestrol was 12.1 per cent more than the mean of the untreated rats and the "significant ratio" was 4.3 (Table 3).

i. Spleen. One of the more consistent results reported after treatment with the natural estrogens is the decrease in the weight and size of the spleen (Cramer and Horning, 1936; Zondek, 1936).

One of the outstanding facts to be seen in Tables 2 and 3 is the extreme variability in the weight of the spleen in the control animals. The values for the coefficient of variation for the uncastrated and castrated groups are 46.3 and 33.2 respectively (Tables 2 and 3). Since only a 6.1 per cent decrease occurred between the mean weights of the treated uncastrated group and their controls no significant change was evident in the value of 0.4 for the "significant ratio" (Table 2). Notwithstanding the extreme variation for the castrated female group a mean weight 42.1 per cent lower than the mean weight of the control group was found after treatment and this gave a "significant ratio" of 4.8 (Table 3).

C. Discussion

The effects of estrogens on the body weight and growth of animals have been reported by numerous investigators (Zondek, 1936; Freudenberger and Clausen, 1937 *A* and *B;* Noble, 1938; Korenchevsky, 1939; Loeser, 1939; Millish, Baer, and Macias, 1940). The elucidation of the mechanism by which these effects are produced would be of great interest and offers a field for further research. Selye and Collip (1936) have suggested that substances which exert a profound effect on the organism may cause a shift in the hormone production of the pituitary gland so that its vital function may predominate with subordination of its gonadotropic and growth hormone production. Zondek (1936) postulated that large dosages of estrone restrain growth by inhibition of the production of growth hormone by the pituitary. Gaarenstroom and Levie (1939) believe that estrone and stilbestrol have a direct action on the epiphyseal junction but that the latter differs from estrone quantitatively and qualitatively in its action. That stilbestrol has a marked depressant action on growth and body weight of laboratory animals has been consistently reported. These findings have been based on studies involving large dosages (1 mg. daily) administered by subcutaneous or intramuscular injection for

relatively short periods. This study confirms the existing reports of the effects of stilbestrol on growth and body weight. It further indicates that the castrated animal is more seriously affected than the uncastrated animal. Possibly the readjustment of the pituitary gland elicited by castration renders this gland less capable of adapting itself to the influences of the administered hormone. Since the castrated females receiving 250 gamma of stilbestrol in 40 per cent alcohol showed a significant weight gain (Table 1, Figure 1) these data obviously need verification. Work now in progress in this laboratory (implantation of pellets of stilbestrol and oral administration of alcohol) would seem to indicate that the above findings can be confirmed.

The effects of continued estrogenic treatment on the estrus cycle of the rat obtained by other workers have already been indicated in this paper. From the data presented one might conclude that although the ovaries were significantly atrophied they nevertheless exerted some control over the estrus cycle. Although the percentage of time in estrus was markedly increased, continuous estrus was not present. The castrated animals on the other hand, did remain in a continuous state of estrus.

A comparison between the uncastrated and castrated female rats of the effects of stilbestrol on the weight of the organs as afforded by this experiment is of some interest. The weights of the thyroid gland, liver, kidney, and spleen of the castrated group were significantly changed while the weights of these organs of the uncastrated group were not significantly changed. With the exception of the liver the weight changes that did occur in the uncastrated group, although not significant by analysis, were in the same direction as the significant changes occurring in the castrated groups. The pituitary gland, uterus, and the thymus weight changes were significant in both groups. The pituitary and uterus were more affected in the castrated series while the thymus of the normal animal sustained a greater weight loss. The only organ which showed an insignificant weight change in both types of animals was the adrenal gland.

Taking cognizance of the fact that age and weight of the animal as well as dosage and period of treatment all influence the results obtained in such an investigation as reported in this paper the findings presented do not differ markedly from those described by other investigators using the natural estrogens.

D. Summary

A total of 50 adult uncastrated virgin female rats received three times weekly for 13 weeks 50 gamma and 250 gamma of stilbestrol in an aqueous alkaline medium. In like manner a total of 24 adult castrated female rats received for seven weeks the same solutions of stilbestrol. Eleven adult uncastrated female rats were likewise treated with 250 gamma of stilbestrol in 40 per cent ethyl alcohol. The effects of the treatment on growth, estrus, and weight of organs were compared with 40 female control rats under like conditions of diet and environment.

It was found that stilbestrol had a pronounced depressant effect on the growth increment of young adult female rats and that castrated females were more affected than uncastrated females.

The length of time in estrus of the uncastrated rats was considerably increased and persistent estrus was established in the castrated rats.

The increase in weight of the pituitary gland and uterus was significant in both the uncastrated and castrated animals. The decrease in weight of the thymus was significant in both types of animals studied. The weights of the thyroid gland, liver, kidneys, and spleen were significantly altered only in the case of the castrated animals. The ovaries were significantly reduced in weight. The weight of the adrenal glands was only insignificantly changed in both the uncastrated and castrated rats.

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[1](*Continued.*) Figure 2 shows the daily per cent of rats in estrus following treatment with 250 gamma of stilbestrol (Group *B*, Table 1). A similar estrous curve was constructed for the 25 rats given 50 gamma of stilbestrol (Group *A*, Table 1) and found to be nearly identical with the curve shown in Figure 2. Therefore one curve adequately represents both groups.

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THE EFFECTS OF l-PROLINE ON PROLIFERATION OF CELLS AND DIFFERENTIATION OF PROTOXYLEM IN ROOTS OF COTTON AND BEAN

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Recent exploratory studies by Hammett and his co-workers have indicated that the naturally occurring amino-acid, l-proline stimulates tissue differentiation in certain animal organisms. In the marine coelenterate, *Obelia,* the application of l-proline in sea-water cultures serves to accelerate the expression of differentiation (Hammett, 1937). Similarly, in the larval growth of *Drosophila melanogaster,* the addition of l-proline tends to shorten the interval of time between successive stadia (Wilson, 1941). Histological and anatomical evidence that l-proline may accelerate tissue differentiation is available from the experimental study of mouse tumors (Hammett, 1941) and from observations on the regeneration of chelae in the hermit crab (Hammett, Hammett, and Goldsmith, 1940).

In view of the foregoing investigations it seemed profitable to determine whether l-proline exerts any influence on the proliferation and differentiation of plant tissue, more particularly since this amino acid has been used as a constituent of culture media (White, 1938). The following investigation provides data which are of use in comparing the action of a specific chemical grouping on both plant and animal organisms. The results of this study are at present essentially qualitative and preliminary.

MATERIALS AND METHODS

In choosing material for testing it was desirable to use some portion or organ of the plant in which the rate of both proliferation and differentiation of cells might be readily measured. In addition it was necessary to have the growing tissue continuously exposed to the proline throughout the duration of each experiment. The roots of seedling plants are well adapted for these purposes. In this study the increase in length of the root was used as an index of prolifera-

tion[1] while the distance from the root cap to the nearest protoxylem element served as the index of the rate of differentiation.

Bean (*Phaseolus vulgaris*) and cotton (*Gossypium hirsutum*) seedlings were chosen as experimental material. Both of these plants produce seeds which are easily obtainable in relatively pure genetic strains, germinate readily, and develop large vigorous primary roots with well delimited hypocotyledonary zones. Cotton seeds from self-pollinated plants were very kindly supplied by Dr. Thomas Kerr of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina. For bean seeds a stringless green pod variety was obtained from W. Atlee Burpee, Inc., Philadelphia, Pennsylvania.

For each experiment the seed coats of the cotton seeds were carefully scarified with a metal file. The scarified seeds were then soaked overnight in distilled water and germinated at 25° ± 1°C on filter paper in moist chambers or in carefully purified sterile sand. By this procedure sufficiently well developed seedlings were obtained within 48 to 60 hours. For their further development, these seedlings were placed on perforated paraffin discs suspended on properly bent glass rods in 150 cc. Pyrex glass beakers. Each beaker supported six seedlings in 100 cc. of solution. Insofar as possible, equal numbers of test and control plants were employed in each experiment. The length of the root of each plant in the test and control solutions was measured carefully before and after every experiment. The cotton roots, after being measured at the completion of the experiment, were cut off 12 or 15 millimeters from the tip, killed and fixed for anatomical study.

The bean seeds were germinated between sheets of moist filter paper after a preliminary overnight soaking in distilled water. When the seedling roots had attained a length of two to four centimeters, the tips were excised at lengths of 6, 10, or 12 millimeters and placed directly in Petri dishes containing the test and control solutions. From six to eight root tips were cultured in each Petri dish. At the completion of the experiment the root tips were again measured before being killed and fixed.

Both the cotton seedlings and bean root tip cultures were placed in

[1]It may well be argued that any increase in length of a root is the result not only of cell division but also of cell elongation. A progressive increase in length, however, is necessarily the result of cell division as well as cell elongation since the zone of cell elongation comprises but a *fraction* of the entire root and is maintained by the successive enlargement of new derivatives of the apical meristem.

a constant temperature incubator and maintained for approximately 24 hours at a temperature of 25° ± 1°C, in the dark.

No attempt was made to maintain strictly sterile conditions during the 24-hours experiments. All glassware and other apparatus, however, was carefully cleaned and sterilized at the beginning of each experiment. Excised root tips were obtained by the use of a flamed lancet and transferred to Petri dishes by means of sterile forceps. Pretreatment of the seeds with chemical fungicides was avoided in order to insure freedom from undue contamination by inorganic ions.

The l-proline used in these experiments was dissolved in a nutrient salt solution. The formula for this solution is the same as that proposed by White (White, 1938), with the exception that iron and zinc salts were omitted in order to avoid the possibility of reaction between heavy metal ions and proline. Sugar was also omitted in order to minimize bacterial contamination. Three concentrations of l-proline were tried, viz., 5/100,000M, 2/100,000M and 1/100,000M. All test solutions were freshly made up from the stock nutrient solution before each experiment. PH readings, determined by the electrometric method, were made for all solutions before and after use. No significant PH changes were noted during the course of the growth experiments.

In order to measure the distance from root cap to the last-differentiated protoxylem element the root tips were cleared by treatment with a 3 to 4 per cent aqueous solution of sodium hydroxide at 55°C for 12 to 18 hours. Lactic acid was also tried as a clearing agent but was not as effective and required much more time to render the tissue transparent. It should be noted that sodium hydroxide causes excessive softening and maceration of thin-walled tissue if allowed to act for a prolonged period at temperatures above 50°C. After the clearing process the root tips were washed in water and examined directly under the microscope without further treatment. Each root tip was placed on a slide and carefully covered with a cover glass to facilitate measuring. Measurements were made with an ocular micrometer at a total magnification of x80.

Results and Discussion

Table 1 presents a summary of the data obtained from the cotton seedling cultures. It can be seen that in the experiments employing a concentration of 5/100,000M proline, growth in length of the roots was greater in the control than in the test plants. At the next concen-

TABLE 1

No. of experiment	Concentration of l-proline	Number of seedlings grown		Mean root length in millimeters				Percentage gain in root length		Number of roots measured		Mean distance of protoxylem from root apex in micrometer units*	
				Test		Control							
		Test	Control	Bef.	Aft.	Bef.	Aft.	Test	Control	Test	Control	Test	Control
1	5/100,000M	34	36	33.5	52.6	34.3	59.6	56.9	74.1	—	—	—	—
3	5/100,000M	26	26	21.1	40.1	20.2	41.0	89.8	102.9	—	—	—	—
5	5/100,000M	20	20	16.0	28.5	17.3	32.3	78.1	86.4	3	4	290	379
7	5/100,000M	30	30	18.0	20.7	19.5	24.5	16.3	25.8	8	10	200	249
9	2/100,000M	18	18	22.9	33.4	25.1	33.7	45.5	34.3	—	—	—	—
11	2/100,000M	30	30	30.6	48.9	30.4	51.8	59.6	70.4	21	21	352	403
13	2/100,000M	37	37	27.1	47.8	25.7	51.5	76.6	97.2	26	19	365	404
15	2/100,000M	26	26	36.7	52.0	38.9	52.7	41.4	34.8	18	18	294	307
17	1/100,000M	20	20	18.9	29.5	18.1	26.6	56.1	47.0	—	—	—	—
19	1/100,000M	25	25	19.5	24.5	20.5	34.1	25.2	66.6	4	5	335	391
21	1/100,000M	35	35	26.6	38.5	28.1	42.4	44.7	50.8	—	—	—	—
23	1/100,000M	31	31	24.4	36.7	26.1	36.9	50.3	41.41	12	12	353	330

*One micrometer unit equals approximately 18.5 u.

tration, viz., 2/100,000M a similar effect was obtained, although it was not as pronounced. With 1/100,000M there was no consistent effect discernible; in two experiments the test plants grew more than the controls and in two similar experiments the reverse occurred.

Preliminary cultures using 1/1000M proline showed a retardation effect somewhat more pronounced than the results obtained with 5/100,000M.

It is evident from the data shown in Table 1 that l-proline definitely retards length growth when applied in concentration of 2/100,000M or stronger. 1/100,000M may, on the other hand, be regarded as a critical concentration at which the effect could not be satisfactorily determined by the experimental methods employed.

In order to determine whether the rate of protoxylem differentiation in root tips is likewise influenced by varying concentrations of proline, measurements were made of the distance from the last-formed protoxylem elements to the root apex.[2] For these measurements the root tips from each growth experiment were used. Five sets of cotton root tips were lost during the clearing process and accordingly the measurements shown in Table 1 are not complete. From the figures available, however, it can be seen that the last-formed protoxylem elements are rather consistently further from the root apex in those roots which increased more in length. This may be interpreted to indicate that in more rapidly growing roots there is a more extensive zone of proliferating and differentiating tissue between the terminal protoxylem and the root apex. Therefore, in general, these measurements indicate merely that it is the total amount of increase in length which seems to determine the length of the zone of cell proliferation and differentiation, and little may be concluded regarding the specific effect of proline on the rate of protoxylem differentiation as distinct from its effect upon length growth.

The general proportionality, in cotton roots, between the rate of increase in length and the distance between protoxylem and root apex is shown also *within* both the control and test series, independent of any comparison between the two. This proportionality further

[2]Root apex refers to the most distal portion of the root cap. The measurements therefore include the root cap and the meristematic zone as well as the region of cell elongation. This method of measuring was followed for practical purposes. Owing to variations in the size of root caps a small but relatively unimportant error is introduced. Measurements from an arbitrary point such as the distal portion of the meristematic zone would, however, introduce a larger error arising from the difficulty of finding the same corresponding reference point in diverse roots.

suggests that it is not possible from the data in Table 1 to assign any specific effect on differentiation to proline, other than that resulting indirectly from its influence upon length growth.

It may be noted that these measurements, indicating the relationship between growth rate and differentiation, are consistent with many observations on the differentiation of vascular tissue in other parts of higher plants. Thus in regions of sustained, rapid growth such as the intercalary meristems of fast-growing monocotyledonous stems and in the rapid spring growth of the cambium of woody plants the rate of tissue differentiation tends to lag behind that of cell proliferation and enlargement.

In view of these facts it would seem that any specific effect of proline on the rate of protoxylem differentiation might be determined by culturing separately in the test and control solutions anatomically comparable root tips. Such material would be less influenced by stored food substances and the general metabolism of the seedling and also would afford greater exposure to the influence of the surrounding medium. Length measurements from these experiments, in addition, would serve as a check upon the length growth data in Table 1 obtained from intact seedlings.

Table 2 gives the data from these cultures of excised bean root tips. It can be seen that the effect of l-proline on the increase in length of the root tips is quite similar to that found in the case of the cotton roots. Here, again, proline shows a general tendency to retard growth in length at concentrations of 5/100,000M and 2/100,000M. Similarly also, at concentrations of 1/100,000M there is not a discernable effect. The actual percentage gain in length of the excised root tips is small, though significantly more in most cases than might result from cell enlargement alone (10-15%). In order to determine whether extensive cell proliferation was actually occurring in the cultures, several sets of test and control root tips were allowed to develop for 48 to 72 hours. In these, the differences in length between the tests and controls were proportionately similar to those determined in the 24-hour experiments, though the absolute differences in length were much greater.

In Table 2 measurements of the distance from the root apex to the last-formed protoxylem elements indicate a reverse trend from that shown in Table 1. Thus, in the excised tips, those test roots in which length growth has been retarded, tend to exhibit a *more extensive* zone of proliferating and differentiating tissue than the

TABLE 2

No. of experiment	Concentration of l-proline	Number of root tips		Mean root tip length mm.				Percentage gain in root tip length		Number of root tips measured		Mean distance of protoxylem from root apex in micrometer units*	
				Test		Control							
		Test	Control	Bef.	Aft.	Bef.	Aft.	Test	Control	Test	Control	Test	Control
2	5/100,000M	34	24	6	7.10	6	7.38	18.3	22.9	23	23	108	100
4	5/100,000M	25	27	10	11.52	10	11.84	15.2	18.4	20	19	80	74
6	2/100,000M	12	12	6	6.91	6	7.12	15.2	18.7	10	10	156	160
8	2/100,000M	26	30	6	6.93	6	7.14	15.5	19.0	14	14	170	131
10	2/100,000M	33	32	12	14.48	12	14.75	20.6	22.9	25	25	151	118
12	2/100,000M	20	20	12	14.13	12	14.05	17.8	17.1	18	18	101	98
14	1/100,000M	28	26	10	12.50	10	13.26	25.0	32.6	19	19	128	101
16	1/100,000M	22	15	10	13.0	10	12.86	30.0	28.6	11	9	96	104
18	1/100,000M	61	66	10	12.02	10	12.07	20.2	20.7	16	10	175	160
20	1/100,000M	36	36	10	11.91	10	11.83	19.1	18.3	14	15	150	175

*One micrometer unit equals approximately 18.5 u.

control roots, in which growth has been more rapid. It might be expected that in the excised root tips, as in the intact cotton roots, the last differentiated protoxylem elements would be further from the root apex in the controls than in the tests, since in general the controls have grown more rapidly and would tend to possess a greater zone of dividing and (or) differentiating cells. Such, however, is not the case and although increase in length is greater in the controls the zone or region of dividing and differentiating cells tends to be shorter than in the test root tips. The cause of this reversed condition is not at all clear although the traumatic effect in excising the root tips may be the most significant factor. However, injury alone, even if considerable, would scarcely be expected to exert a differential effect on the test and control root tips.

It should be noted in Table 2 that *within* the control series of bean root tips there is no consistent relationship between increase in length of the root and the distance from the root apex to protoxylem. The absence of such relationship within the controls tends to vitiate conclusions which may be drawn from these data concerning the specific effect of proline on the differentiation of protoxylem.

In general the excised root tip cultures supplement the results of the cotton seedling cultures. Both sets of experiments indicate that proline tends to retard the length growth of roots. There is a slight indication that this retarding influence is roughly proportional to the molecular concentration of l-proline in solution. Neither set of experiments shows definitely whether or not proline exerts a specific influence on the rate of protoxylem differentiation as distinct from its influence on length growth. Traumatic effects may well invalidate putative conclusions drawn from the excised root tip experiments.

It should be emphasized that this study is essentially qualitative and indicates a general *trend* in the response of roots to proline. Much further investigation is necessary to determine the various histological changes, the specific mechanism, and the quantitative influence of proline on root growth.

Summary

The qualitative influence of effective concentrations of l-proline on the growth of cotton seedling roots and excised bean root tips has been determined.

Entire cotton seedlings and excised bean root tips were grown for 24-hour periods, under controlled conditions, in nutrient salt solutions.

The amount of increase in the length of the roots during the 24-hour periods was adopted as an index of cell proliferation. The distance from the terminus of the root cap to the last differentiated protoxylem elements was taken as an index of the rate of cell differentiation.

In cotton roots, as well as in excised bean root tips 5/100,000M and 2/100,000M concentrations of proline tended to retard growth in length. 1/100,000M proline had no apparent influence. There is some evidence that this retarding influence is roughly proportional to the molecular concentration of proline in solution.

No clear-cut evidence was obtained from either set of experiments to show a specific effect of proline on the rate of protoxylem differentiation. Possible traumatic effects in excised root tips must be taken into consideration in drawing conclusions from such material.

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INFLUENCE OF pH ON THE PROLIFERATION OF NOSTOC MUSCORUM*

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Since a series of experiments dealing with the transformation of lifeless into living has been planned using Nostoc, it was feasible to learn something of its pH range. Nostoc has been chosen because of its lack of an organized nucleus. In recent work, in which different hydrogen ion concentrations of Bristol solutions were used, it was found that this alga proliferated very little, if any, at pH ± 6. This affirms the work of Allison, Hoover, and Morris (1937) who used a $NaNO_3$ free solution. In their work, they found that the optimum range was from 7.0 to 8.5, and that its upper limit for growth was approximately 9, and its lower limit between pH 5.5 — 5.7. Original Bristol's solution containing $NaNO_3$, when made up, has a pH of 4.8 — 5.0, and it was found that a great variety of blue greens grew on it, including Nostoc muscorum. Since Allison *et al.* did not determine the optimum pH or whether Nostoc can grow at a pH lower than 5.5, if given combined nitrogen, a series of experiments was devised to determine proliferation over a wide range with and without $NaNO_3$.

The material and method used is similar to that described elsewhere (Walp, 1937; Hammett and Walp, 1939; Walp, 1941). The Allison, Hoover, and Morris method was entirely different from the aliquot method of determining cell number increase, since it consisted in measuring the amount of nitrogen fixed by the cells. The aliquot method is quantitative and deals with relatively fewer cells than the chemical method of Allison *et al.* In all experiments, except those at pH 4.5, there was no noticeable change of pH at the end of an experiment.

The pH of Bristol solution was modified by using *mono, di,* or *tri* potassium phosphate, as shown in the table. Table 1 contains the results of the experiments grown in $NaNO_3$ solution. It gives the

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TABLE 1
OPTIMUM pH FOR GROWTH

Experiment number	Hours run	Number of sets			Total Number Cells						Percentages				
					Acid		Neutral		Basic		Increase			Difference	
		Acid	Neut.	Basic	Start	End	Start	End	Start	End	Acid	Neut.	Base	N-A	N-B
1	48	12	13	12	1369	1680	1699	2355	1405	1740	22.7	38.1	23.8	+15.4	+14.3
2	96	7	7	8	929	1877	1074	3260	1423	3135	102.0	203.5	120.3	+101.5	+83.2
3	96	8	5	7	1419	1695	795	1266	1136	1643	19.5	59.2	44.6	+39.7	+14.6
4	48	9	9	9	1019	1201	774	1106	865	1083	17.9	44.8	25.2	+26.9	+19.6

Acid Medium, pH±5.4, KH_2PO_4 0.781 g., KCl 0.428 g., $NaNO_3$ 1.0 g., $MgSO_4$ 0.3 g., NaCl 0.1 g., $CaCl_2$ 0.1 g., $FeCl_3$ trace, H_2O 1000 cc.

Neutral Medium, pH±6.9, K_2HPO_4 0.650 g., KH_2PO_4 0.273 g., KCl 0.150 g., $NaNO_3$ 1.0 g., $MgSO_4$ 0.3 g., NaCl 0.1 g., $CaCl_2$ 0.1 g., $FeCl_3$ trace, H_2O 1000 cc.

Alkaline Medium, pH±7.8, K_2HPO_4 1.0 g., $Na.NO_3$ 1.0 g., $MgSO_4$ 0.3 g., NaCl 0.1 g., $CaCl_2$ 0.1 g., $FeCl_3$ trace, H_2O 1000cc.

pH determined by Beckmann pH meter.

total number of cells started in Bristol's acid, neutral, and alkaline solution for each experiment; their percentage increase; and the difference between the neutral and acid percentages, as well as those between the neutral and basic percentages.

From the table we find the following: (*a*) the cells proliferate best at neutral (pH 6.9); (*b*) proliferation is greater in alkaline (pH 7.8) than in acid (pH 5.4). This, with the exception of being able to grow them in pH 5.4, agrees with the work of Allison *et al.*

Since Allison didn't use $NaNO_3$, supposing that Nostoc was able to fix its own N from the air, another set of experiments was set up, this time leaving out $NaNO_3$ from our solution. Table 2 gives the results of this experiment. From it the data would indicate that, without $NaNO_3$, it doesn't make any difference whether the medium is acid, neutral, or alkaline.

Knowing that Nostoc will grow in the low pH 5.4 with or without $NaNO_3$, an attempt was made to grow it at still lower pH's, viz., pH 5, pH 4.5, and pH 3.5. No growth occurred at pH 3.5, excellent growth occurred at pH 4.5, but the pH continually rose to pH 5. Then several experiments were done at pH 5 using both Walp's and Allison's strains of Nostoc to determine if they might be different. Table 3 gives the results. The first four experiments are those using Walp's culture, and the last two using Allison's culture; in all cases there was at least a 20 per cent increase in cell number at pH 5.

There is no question that pH affects the rate of cell proliferation of Nostoc muscorum when grown in a $NaNO_3$ solution. Walp (1941) and Allison (1937) have shown that growth is retarded or stopped at pH ± 6. Using $NaNO_3$, the direction of influence is apparently determined by the alkalinity and acidity of the culture media. Neutral appears to be the best medium for cell proliferation. Alkaline solution is better than acid. But when it comes to growing Nostoc without $NaNO_3$, it was found that hydrogen ion concentration didn't make much difference as far as cell proliferation was concerned. No explanation can be offered for this. It was found that both Walp's and Allison's culture of Nostoc muscorum could be grown as low as pH 5 in nitrate-free cultures. pH 3.5 killed both cultures; pH 4 to 4.9 showed some growth, but culture changed the pH even in the aliquots.

The only explanation for the difference in the extremes is that the age of the algae may have had something to do with the fixation of nitrogen.

TABLE 2

OPTIMUM pH FOR GROWTH: $NaNO_3$ FREE MEDIA

Experiment number	Hours run	Number of sets			Total Number Cells						Percentages				
					Acid		Neutral		Basic		Increase			Difference	
		Acid	Neut.	Basic	Start	End	Start	End	Start	End	Acid	Neut.	Base	N-A	N-B
1	72	10	11	11	1210	1604	1376	1546	1559	1835	32.6	12.4	17.7	—20.2	—5.3
2	48	7	7	8	828	1007	691	977	796	1006	21.6	42.4	26.4	+20.8	+18.0
3	120	10	11	11	1585	1626	1817	1979	1827	1845	2.6	8.9	1.0	+6.3	+7.9
4	96	13	11	12	1547	1954	1110	1342	1324	1625	26.3	20.9	22.7	—5.4	—1.8
5	96	10	11	11	2186	3605	1857	2730	1904	3362	64.9	47.0	76.6	—17.9	—29.6

Acid Medium pH±5.4, KH_2PO_4 0.758 g., K_2HPO_4 0.03 g., KCl 0.415 g., $MgSO_4$ 0.3 g., NaCl 0.1 g., $CaCl_2$ 0.1 g. $FeCl_3$ trace, H_2O 1000 cc.

Neutral Medium pH±6.9, KH_2PO_4 0.273 g., K_2HPO_4 0.650 g., KCl 0.150 g., $MgSO_4$ 0.3 g., NaCl 0.1 g., $CaCl_2$ 0.1 g., $FeCl_3$ trace, H_2O 1000 cc.

Alkaline Medium pH±7.8, K_2HPO_4 1.0 g., $MgSO_4$ 0.3 g., NaCl 0.1 g., $CaCl_2$ 0.1 g., $FeCl_3$ trace, H_2O 1000 cc.

pH determined by Beckmann pH meter.

TABLE 3

GROWTH IN ACID: MEDIUM pH 5 WITHOUT $NaNO_3$

Experiment number	Hours run	Number of set	TOTAL number cells Start	End	Percentage increases
1	24	13	2497	3726	49.2
2	48	13	2039	3088	51.4
3	24	10	1456	1765	21.2
4	96	10	1311	1787	36.3
5	144	11	1402	2155	53.7
6	96	10	1287	2118	64.6

Bristol's culture medium:
pH 5, KH_2PO_4 0.781 g., KCl 0.428 g., $MgSO_4$ 0.3 g., NaCl 0.1 g., $CaCl_2$ 0.1 g., $FeCl_3$ trace, H_2O 1000 cc., HCl q.s.

SUMMARY

1. Four experiments with Nostoc muscorum show that neutral Bristol's with $NaNO_3$ is optimum for cell proliferation and that an alkaline solution is slightly better than an acid medium.

2. pH didn't appear to have any effect upon rate of cell proliferation when $NaNO_3$ was omitted from acid, alkaline, and neutral solutions.

3. Nostoc muscorum can be grown without difficulty at pH 5. The lowest range is between pH 5 and pH 3.5, the latter killing it.

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THE WEIGHTS OF THE BRAIN AND OF ITS PARTS, AND THE WEIGHT AND LENGTH OF THE SPINAL CORD IN THE DOG

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Many biometric studies of the human brain have been made, not only on individuals of various mental abilities but also of various races, and more recently the various divisions of the brain and its finer structure have received more attention quantitatively. Weights of the brain were compared to stature as early as 1836 by Parchappe. The brain of the albino rat has likewise been studied quantitatively very carefully in both its gross and microscopic aspects, but no attempt will be made to review the abundant literature in these fields. The brain of the dog has not received as much attention. There are several papers giving the weights of the entire dog brain (Hrdlicka, '07, Pezard, '36, and Crile and Quiring, '40). Kappers has studied the weights of the cortex ('26) and the cerebellum ('27) in various animals including the dog. The weights of the brain and of five of its divisions and of the cord for 11 dogs were included in a paper by Latimer ('24). Winterstein ('26) has gathered the available data on the water content and chemical analyses of various brains including the dog brain. There still seems to be the need of a statistical study on a more adequate number of dog brains and spinal cords.

The weights and the percentage weights of the brain and of seven of its divisions and the weight and length of the spinal cord from 321 dogs will be given. Then the variability of the various parts of the central nervous system will be presented and also the sex differences. Empirical formulae for determination of the weights of any of the divisions of the central nervous system from either body weight or body length will be presented as a more satisfactory method of estimating these weights than by the use of the averages or the percentages. The asymmetry of the two halves of the forebrain will be discussed, and then the correlations of the various parts of the central nervous system will be given.

MATERIALS AND METHODS

The brain, spinal cord, and the hypophysis were removed from 162

male and 159 female adult dogs. The dogs were those used for experiments in the physiology laboratory in which the animal was sacrificed after an experiment lasting only one laboratory period or less. In no case were animals used after prolonged experiments which would affect the general condition of the animal. All of the dogs were adults, as judged by the teeth and the ossification of the bones, and all were in good health. They were of all breeds and many of no recognizable breed, but they undoubtedly represent a good cross section of the animals used in routine laboratory experiments. Some came from the country and others from towns and cities. While the dogs were being used for this work all of the dogs from a certain laboratory section were taken unless they were immature or showed signs of disease. Toward the end only females were taken. The central nervous systems were taken from the dogs at various times from October, 1936, until July, 1939.

The dogs were weighed on a platform balance sensitive to ¼ lb. at the beginning of the experiment and these weights changed to the metric system were used, as it would have been impossible to have reweighed the animals after the experiment in which frequently more or less blood was lost. The dog was placed back up and the skin and most of the dorsal musculature was rapidly removed very soon after the killing of the animal. The head was severed from the vertebral column by an incision passing close to the occipital condyles which cut the cord from the medulla at approximately the level of the first root fila of the first spinal nerve. The laminae of the vertebrae were removed and the dura cut down the mid-dorsal line and turned back and while still attached to the vertebral column the length of the cord was measured from the cut edge to the beginning of the filum terminale by means of a flexible steel tape graduated to millimeters. Then the cord was removed and placed in a moist chamber together with the brain and hypophysis until taken to the laboratory and weighed.

The skin, muscles, the top of the skull, and the dorsal part of the dura were next removed and the brain allowed to fall gently into the palm of the hand from the inverted skull. The olfactory lobes were carefully separated from the cribiform plate and each pair of nerves were cut in order. The stalk of the hypophysis was cut as the brain was removed and later the hypophysis itself was carefully removed and its weight has been reported in a separate paper (Latimer, '41). The meninges, blood vessels, and nerves of some length were left on the brain and cord until they were prepared for weighing. Several medical

students assisted in this part of the work, but the following final preparation of the parts for weighing and the separating of the brains into their several divisions as well as the weighing was all done by the author.

The dura and all large blood vessels were removed, all nerves were cut close to the cord, and then it was weighed in a glass stoppered weighing bottle on a chemical balance sensitive to a tenth of a milligram.

The brain was freed of all blood vessels, all nerves were cut close to the brain, and then it was divided in a manner similar to that employed in the study of the cat brain (Latimer, '38a and '38b). The pia mater is included in the brain weights, but all of the ventricles were emptied. The olfactory bulbs were separated from the rest of the brain at the very evident narrowing to form the olfactory tract. Next the cerebellum was removed by cutting the peduncles on a level with the margin of the walls of the medulla. This left the pons to be weighed with the medulla. The medulla and midbrain were separated at the isthmus, a very evident constriction. Then the separation of mesencephalon and diencephalon was made by an incision beginning dorsally on each side just anterior to the two anterior corpora quadrigemina and inferiorly cutting through just posterior to the stalk of the hypophysis. This incision was not a straight transverse cut but rather a very obtuse wedge shape with the mid portion the farthest anterior. The incisions were made part way through the brain stem beginning at both the upper and the ventral surfaces of this region and meeting in the middle. It was felt that this was a little more accurate method of making this, the most difficult separation of the parts of the brain. In nearly half of the dogs the remaining hemispheres and diencephalon were split into a right and a left half and these weighed separately. In these dogs the weight of the hemispheres and diencephalon is the sum of the weights of the two halves. The parts were put in glass stoppered weighing bottles as soon as dissected and weighed in the same manner as the cord. The total brain weight is the sum of the parts. It was felt that this would prevent additional handling of the brain and exposure to drying. All of the weights and measurements were made on the brain in the fresh condition.

The formulae used in the determination of the statistical constants are those usually so employed and are given by Dunn ('29). All of the computations were carefully checked, most of them by two workers.

First of all I wish to express my gratitude to Dr. O. O. Stoland

and to the other members of the Physiology Department for their kindness in permitting the use of this material, and especially to Mr. Fred Johnson who was so patient with us while we gathered this material. I also wish to express my appreciation of the careful assistance of the following medical students who helped with the removal of the brains and cords from the dogs; Mr. Edgar P. Sereres, Mr. Lelond Holbert, Mr. Milton H. Noltensmeyer, and Mr. Robt. R. Remsberg. To Miss Edna Mae McConnell and Mr. Douglas J. Malone I wish to express my appreciation of their careful, accurate work in assisting with the statistical part of this study.

Weights

The averages of the absolute and percentage weights and linear dimensions together with their coefficients of variation and the significant ratios are shown in Table 1. As has been stated above, this group of dogs is composed of all kinds and sizes, and with a range of body weight in the males from 4 to 30 kilograms and in the females, 4 to 24 kilograms, these averages are not very reliable, except of course for dogs of average size. Empirical formulae will be given later on for the more accurate estimation of the weights of the parts of the central nervous system. The data for the body weight and body length are given in the last two lines of Panel *A*.

The body weight is the most variable measurement in both sexes, followed by the weight of the spinal cord. The body length and the cord length, especially in the males, have very similar coefficients of variation, which is to be expected. In the males, the weight of the entire brain is the least variable of all the ponderal measurements, while in the females there are three of the divisions of the brain slightly less variable. In the description of the methods of dissection of the brain parts it was suggested that the most difficult separation was between the relatively small mesencephalon and the much larger forebrain. Thus any irregularity in making this cut should be most evident in the weights of the midbrain. In the males, in Panel *A*, Table 1, the variability of the midbrain is fifth in order of increasing variability but still it is grouped with the parts of the hindbrain and just next in order above the medulla. In the females however it is the least variable of the seven parts of the brain. Hence it seems that the method of dividing the brain into its parts and especially this most difficult cut was done fairly well as shown by these coefficients of variability. The average of the first 10 coefficients of variation in

TABLE 1

MEASUREMENTS AND COEFFICIENTS OF VARIATION OF THE BRAIN AND OF ITS DIVISIONS, AND OF THE SPINAL CORD IN THE DOG

	162 male dogs		159 female dogs		Diff.
	Average weight or length	Coefficient of variation	Average weight or length	Coefficient of variation	*PE* diff.
	A. *Weights and linear measurements*				
Brain	76.918 ± 0.580	14.24 ± 0.54	73.000 ± 0.570	14.60 ± 0.56	4.82
Olfactory bulbs	1.542 ± 0.017	21.41 ± 0.84	1.473 ± 0.018	23.31 ± 0.93	2.72
Hemispheres and dien.	61.975 ± 0.480	14.61 ± 0.56	58.967 ± 0.477	15.13 ± 0.59	4.44
Prosencephalon	63.517 ± 0.492	14.62 ± 0.56	60.438 ± 0.491	15.18 ± 0.59	4.43
Mesencephalon	2.045 ± 0.018	16.31 ± 0.63	1.915 ± 0.014	13.52 ± 0.52	5.78
Cerebellum	7.369 ± 0.061	15.59 ± 0.60	6.954 ± 0.053	14.20 ± 0.55	5.15
Medulla	3.986 ± 0.036	16.94 ± 0.65	3.691 ± 0.035	17.65 ± 0.69	5.91
Rhombencephalon	11.355 ± 0.091	15.12 ± 0.58	10.645 ± 0.083	14.51 ± 0.56	5.78
Cord weight	14.437 ± 0.181	23.63 ± 0.94	12.955 ± 0.180	25.95 ± 1.05	5.81
Cord length (cm.)	51.09 ± 0.37	13.41 ± 0.52	48.02 ± 0.40	15.33 ± 0.61	5.60
Body weight (kilos)	11.00 ± 0.26	44.75 ± 1.98	8.93 ± 0.22	46.13 ± 2.08	6.07
Body length (cm.)	81.9 ± 0.58	13.40 ± 0.51	76.8 ± 0.61	14.77 ± 0.57	6.03
	B. *Percentages of body weight*				
Brain	0.795 ± 0.013	31.76 ± 1.30	0.928 ± 0.015	30.23 ± 1.24	6.70
Olfactory bulbs	0.016 ± 0.00027	32.23 ± 1.33	0.018 ± 0.00028	28.33 ± 1.15	6.43
Hemispheres and dien.	0.640 ± 0.011	32.44 ± 1.34	0.751 ± 0.012	30.53 ± 1.26	6.82
Prosencephalon	0.656 ± 0.011	32.37 ± 1.33	0.769 ± 0.013	30.44 ± 1.25	6.93
Mesencephalon	0.021 ± 0.00037	33.09 ± 1.37	0.025 ± 0.00044	33.55 ± 1.40	5.91
Cerebellum	0.076 ± 0.0012	30.95 ± 1.27	0.089 ± 0.0015	32.00 ± 1.33	6.87
Medulla	0.041 ± 0.00063	29.33 ± 1.20	0.047 ± 0.00075	30.13 ± 1.24	6.13
Rhombencephalon	0.117 ± 0.0018	29.81 ± 1.21	0.135 ± 0.0023	31.10 ± 1.28	6.16
Cord weight	0.144 ± 0.0019	25.35 ± 1.01	0.160 ± 0.0022	26.00 ± 1.05	5.50
Cord length	0.527 ± 0.0085	29.62 ± 1.23	0.603 ± 0.0091	27.38 ± 1.09	6.10
	C. *Percentages of brain weight*				
Olfactory bulbs	2.00 ± 0.015	14.42 ± 0.55	2.01 ± 0.015	13.52 ± 0.52	0.47 (♀)
Hemispheres and dien.	80.54 ± 0.068	1.58 ± 0.059	80.72 ± 0.075	1.74 ± 0.066	1.74 (♀)
Prosencephalon	82.54 ± 0.064	1.46 ± 0.055	82.72 ± 0.071	1.61 ± 0.061	1.84 (♀)
Mesencephalon	2.67 ± 0.013	9.39 ± 0.35	2.64 ± 0.015	10.58 ± 0.40	1.76 (♂)
Cerebellum	9.60 ± 0.045	8.75 ± 0.33	9.57 ± 0.045	8.75 ± 0.33	0.49 (♂)
Medulla	5.19 ± 0.027	9.74 ± 0.37	5.07 ± 0.030	10.90 ± 0.42	2.98 (♂)
Rhombencephalon	14.78 ± 0.058	7.36 ± 0.28	14.63 ± 0.062	7.96 ± 0.30	1.77 (♂)

Panel *A* is 16.94 for the females and 16.59 for the males or, the females average a little over two per cent more variable than the males.

Panel *B* shows these weights and linear dimensions expressed as percentages of the body weight. The averages of these coefficients are 30.70 for the males, and 29.97 for the females, or the parts of the nervous system of the males are 1.85 times more variable when expressed as percentages of the body weight and for the females the ratio is 1.77 times. The absolute weights are slightly more variable in the females, but the percentages are more variable in the males by 2.4 per cent.

The last column gives the significant ratios, and all but one of the absolute weights and both of the linear dimensions are significantly greater in the males but all of the percentages of the body weight are significantly greater in the females. The only measurement in Panels *A* and *B* which does not show a significant difference is the weight in grams of the olfactory bulbs, and it is almost significant.

The weight of the cord is next to the body weight in variability in Panel *A*, but when the weights are reduced to percentages of the body weight, the lowest coefficient of variation is that of the cord in both sexes as shown in Panel *B*. In other words the weight of the cord tends to follow the variations in the body weight more than the weight of the brain or any of its parts. The medulla follows the body weight the best of any of the parts of the brain as shown by these coefficients in Panels *A* and *B*. The olfactory bulbs are the most variable of the parts of the brain in absolute weight in both sexes. A part of this may be due to the difficulty in removing all of the bulbs.

The weights of the parts of the brain expressed as percentages of the total brain weight are shown in Panel *C* of Table 1. Here we find the lowest coefficients of variation. The average for the males is 7.52 per cent and for the females, 7.88 per cent, or 4.8 per cent greater than for the males. It will be shown later that these percentages of the total brain weight do not change with the size of the dog and hence these percentages shown in Panel *C*, carry more weight than the percentage weights in Panel *B* or the absolute weights in Panel *A*. The last column again shows the ratios, none of which are significant although the medulla is almost significantly greater in the males. Although these are not significantly different, it is interesting to see that the first three, or the prosencephalon and its divisions, are greater in the females and the mesencephalon and the rhombence-

phalon are greater in the males. This posterior part of the brain is more intimately connected with the cord and also with the control of the body which of course is greater in the males, in size at least, if not in activity as well.

The earlier data on the weight of the dog nervous system (Latimer, '24) are not easily comparable with this series because the sexes are not separated and because of the small number of specimens. There is a much greater degree of variability in body weight and in the weights of all the parts of the nervous system in the smaller series. In this earlier series the brain stem (mesencephalon and medulla) seem to be a little lighter and they also form a somewhat smaller percentage of the total brain weight and the prosencephalon is heavier and forms a larger percentage of the brain weight, but in a series of only 11 doges these variations are not significant. Kappers ('26) gives the weight, but not the sex, of two dog brains as 78.5 and 77.7 grams. These are very close to the average brain weights in my dogs. Crile and Quiring ('40) give the total brain weight for ten male and eight female dogs. These weights expressed as percentages of the body weight are slightly lower than in my series and also they are slightly lower in the females than in the males.

In general, the weight of the brain and its parts, the weight and length of the cord and the body weight and length are with one exception significantly greater in the males. They average a little over two per cent more variable in the females. When these weights of the nervous system are expressed as percentages of the body weight, they are much more variable, with the greatest average variability in the males. All of the parts of the central nervous system, as percentages of the body weight, are significantly greater in the females. The parts of the brain expressed as percentages of the total brain weight are the most constant and there is no significant sex difference in their percentages although there is a suggested greater percentage in the weight of the forebrain and its parts in the females, and of the midbrain and the rhombencephalon in the males.

Empirical Formulae

The average weights and the average percentages given in Table 1 are obviously valid for dogs of approximately average body weight; but in any random selection of dogs, such as this series, a large proportion would be much above or below these averages and to better determine the weights of the various divisions of the central nervous sys-

tem, empirical formulae have been developed by means of which the weight of any part of the central nervous system can be computed from either the body weight or the body length. This is the same method which was used for the central nervous system of the cat (Latimer, '40).

The weights of the brain and of its seven divisions, the weight and the length of the cord were each plotted against body weight and body length. The body weights were averaged for each two kilograms, the body lengths for each 10 centimeters increase in length, and the weights of the parts of the central nervous system were likewise averaged for each group and the common point for each two kilograms increase in body weight or for each 10 centimeters increase in body length were plotted and regression lines drawn through these points. Empirical formulae were developed for these regression lines. They were all straight lines and to save space they are not shown. The individual cases follow the lines with only a moderate amount of scatter.

There is a sex difference in the weights or length of all but one of the parts of the central nervous system and so the parts are plotted separately for the two sexes. The following formulae for the males, plotted on body weight, are valid between 4 and 26 kilos of body weight. In each of these formulae, Y represents the weight in grams or the length in centimeters of the part and X, the body weight in kilograms.

Brain,	$Y = 1.69X + 58.44$
Olfactory bulbs,	$Y = 0.046X + 1.066$ (4-18 Kilos, and from 18-26 K. average 1.93 grams)
Hemispheres and diencephalon,	$Y = 1.37X + 47.52$
Prosencephalon,	$Y = 1.37X + 49.32$
Mesencephalon,	$Y = 0.037X + 1.612$
Cerebellum,	$Y = 0.158X + 5.612$
Medulla,	$Y = 0.124X + 2.584$
Rhombencephalon,	$Y = 0.26X + 8.46$
Cord weight,	$Y = 0.61X + 7.76$
Cord length,	$Y = 1.37X + 35.92$

Using the same symbols the formula for the body length plotted on body weight is as follows:

$Y = 2.02X + 59.92$ from 4 to 26 kilos of body weight.

The average percentage deviations of the calculated from the observed values for each two kilograms increase in body weight were

determined and for the males they are as follows: brain, 1.72; olfactory bulbs, 4.26; hemispheres and diencephalon, 1.97; prosencephalon, 1.91; mesencephalon, 1.32; cerebellum, 1.89; medulla, 2.37; rhombencephalon, 1.88; cord weight, 2.04; cord length, 1.84 and for the body length on body weight, 1.40 per cent.

Similar formulae for the weights of the parts of the central nervous system of the female dogs plotted on body weight from 4 to 21 kilograms of body weight are given below. Y again represents the weight or length of the part and X, the body weight in kilograms.

Brain,	$Y = 2.06X + 54.76$
Olfactory bulbs,	$Y = 0.074X + 0.824$
Hemispheres and diencephalon,	$Y = 1.52X + 45.92$
Prosencephalon,	$Y = 1.72X + 45.12$
Mesencephalon,	$Y = 0.045X + 1.53$
Cerebellum,	$Y = 0.176X + 5.346$
Medulla,	$Y = 0.117X + 2.662$
Rhombencephalon,	$Y = 0.3X + 8.04$
Cord weight,	$Y = 0.68X + 6.93$
Cord length,	$Y = 1.67X + 32.92$
Body length (cm.),	$Y = 2.7X + 52.45$

The average percentage deviation of the calculated from the observed averages for these formulae are as follows: brain, 2.48; olfactory bulbs, 6.44; hemispheres and diencephalon, 2.53; prosencephalon, 2.72; mesencephalon, 1.89; cerebellum, 1.95; medulla, 4.01; rhombencephalon, 2.14; cord weight, 4.46; cord length, 2.38 and body length 2.52 per cent.

The formulae for these same parts of the central nervous system plotted on body length in centimeters are given below. Again Y represents the weight in grams or length in centimeters, and X now represents the body length measured in centimeters. These formulae are valid from 65 to 120 centimeters of body length for the males.

Brain,	$Y = 0.79X + 12.65$
Olfactory bulbs,	$Y = 0.022X + 0.26$
Hemispheres and diencephalon,	$Y = 0.62X + 11.60$
Prosencephalon,	$Y = 0.62X + 13.40$
Mesencephalon,	$Y = 0.018X + 0.57$
Cerebellum,	$Y = 0.076X + 1.19$
Medulla,	$Y = 0.052X + 0.26$
Rhombencephalon,	$Y = 0.13X + 0.82$
Cord weight,	$Y = 0.27X - 7.35$
Cord length,	$Y = 0.621X$

The average percentage deviations of the calculated from the ob-

served values for the 10 centimeter groups are as follows: brain, 2.89; olfactory bulbs, 3.31; hemispheres and diencephalon, 2.85; prosencephalon, 2.86; mesencephalon, 1.96; cerebellum, 2.92; medulla, 2.47; rhombencephalon, 2.55; cord weight, 3.76 and cord length, 2.59 per cent.

The formulae for the females plotted on body length in centimeters, from 55 to 110 centimeters of body length are given below. Again Y represents the weight in grams or the length in centimeters of the part and X now represents the body length measured in centimeters.

Brain,	$Y = 0.72X + 17.70$
Olfactory bulbs,	$Y = 0.022X - 0.21$
Hemispheres and diencephalon,	$Y = 0.59X + 13.85$
Prosencephalon,	$Y = 0.58X + 16.10$
Mesencephalon,	$Y = 0.016X + 0.71$
Cerebellum,	$Y = 0.063X + 2.16$
Medulla,	$Y = 0.042X + 0.48$
Rhombencephalon,	$Y = 0.099X + 3.096$
Cord weight,	$Y = 0.26X - 6.49$
Cord length,	$Y = 0.63X - 0.11$

The average percentage deviations for the above formulae are: brain, 2.14; olfactory bulbs, 1.97; hemispheres and diencephalon, 2.47; prosencephalon, 2.28; mesencephalon, 1.75; cerebellum, 197; medulla, 2.31; rhombencephalon, 1.54; cord weight, 2.67, and cord length, 1.74 per cent.

This attempt to relate brain weight and body size is not new for it has been done many times for the human brain and Dubois ('14) has attempted to relate the total brain size and body size in various animals, but so far as is known it has never been attempted before for the dog nor for the parts of the dog brain.

The percentage weights of the brain, of its divisions and of the weight and length of the cord were plotted on body weight for both sexes in the same manner as the absolute weights. The averages were determined as described above for each two kilograms of body weight and through these points smoothed curves were drawn. All of the curves for both the males and the females were similar and to save space only a part of the curves for the males are shown in Figure 1. The smaller divisions of the brain are not shown but they may be determined easily by using the percentages of the entire brain weight (Table 1, Panel *C*) and the brain weight may be checked from Figure 1. The curves for the females resemble those of the males although they drop a little more rapidly.

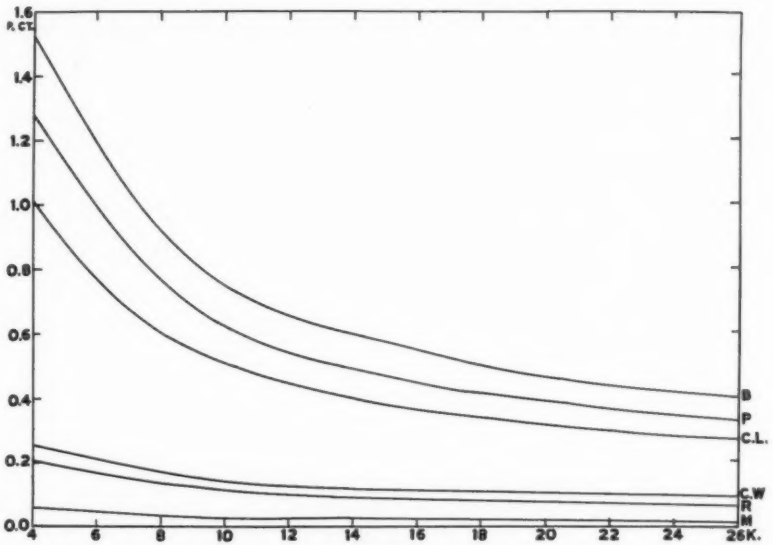

FIGURE 1

WEIGHTS OF SOME OF THE PARTS OF THE CENTRAL NERVOUS SYSTEM AS PERCENTAGES OF THE BODY WEIGHT AND PLOTTED ON BODY WEIGHT

The percentages are shown at the left and the body weights in kilograms at the bottom. The curves from above down are as follows; *B* represents the percentages of the total brain weight; *P* represents the percentages of the prosencephalon; *C.L.*, the cord length; *C.W.*, the cord weight; *R*, the rhombencephalon, and *M* represents the percentages of the mesencephalon.

The percentage values for each division of the central nervous system for both the males and the females for the smallest and the heaviest groups are given in Table 2 and the last column for each sex gives the percentage decrease. The smaller decreases are found in the cord and the hindbrain, parts which are more closely connected with the fundamental activities of the body. These percentage decreases are greater for the males in Table 2 but if we take into consideration the greater range of body weight, the percentage decrease per kilogram of body weight is greater in the females, or the curves decrease a little more rapidly in the females. The percentage decrease per kilo of increase in body weight varies for the different parts of course, but the average for the males is 3.4 per cent and for the females it is 4.0 per cent.

The weights of the seven divisions of the brain were reduced to

TABLE 2
CHANGES IN THE PERCENTAGES OF THE BODY WEIGHT

	Male dogs			Female dogs		
	Per cent at 5.08 K.	Per cent at 25.19 K.	Percentage decrease	Per cent at 5.19 K.	Per cent at 20.46 K.	Percentage decrease
Brain	1.334	0.405	69.64	1.250	0.459	63.28
Olfactory bulbs	0.0254	0.0078	69.29	0.0230	0.0104	54.78
Hemispheres and dien.	1.090	0.327	70.00	1.012	0.367	63.74
Prosencephalon	1.115	0.335	69.96	1.035	0.378	63.48
Mesencephalon	0.0354	0.0110	68.93	0.0345	0.0121	64.93
Cerebellum	0.1197	0.0375	68.67	0.1216	0.0425	65.05
Medulla	0.0634	0.0221	65.14	0.0633	0.0257	59.40
Rhombencephalon	0.1831	0.0596	67.45	0.1849	0.0682	63.12
Cord weight	0.2152	0.0916	57.43	0.204	0.102	49.72
Cord length	0.862	0.269	68.79	0.812	0.313	61.48

percentages of the total brain weight and the averages given in Panel *C* of Table 1. These individual percentages were plotted on body weight as was done for the percentages of the body weight and it was interesting to see that for each of the seven divisions of the brain for both the males and the females the resulting curves were horizontal straight lines, or the proportions of the brain are constant throughout the entire range of body weight in both sexes. Hence by using the percentages given in Panel *C* of Table 1, one can find the weight of any division of any sized dog brain whose entire weight is known and is within the given limits. Not only are these percentages constant throughout the entire range of body weight but there is little if any sex difference. The last column in Panel *C*, Table 1, gives the significant ratios and the only part which approaches a significant ratio is the ratio of 2.98 for the medulla. This is so nearly significant that we very likely should say that this part alone is significantly greater in its percentage of the total brain weight in the males. Kappers ('26) has determined the weight of the hemispheres in two dogs and he reports 86.8 and 83.3 per cent of the total brain weight. However he included what I have listed separately as midbrain and so with this added to my data, the males in my series would average 85.21 per cent and the females 85.36 per cent and the average of his two cases is 85.05 which means that his two cases fall within the normal range of variation of my series.

In general the parts of the central nervous system in both the males and in the females are relatively heaviest in the smallest dogs. The cord and the hindbrain decrease less in percentage of body weight than the other parts of the brain. The relative proportions of the

brains in both sexes are remarkably constant throughout the entire range of body weight. This is shown by the low coefficients of variation shown in Panel *C* of Table 1, and also by the straight horizontal lines when these percentages of the brain weight are plotted on body weight. These percentages in Panel *C* are valid, within limits of individual variation of course, for the entire range of body weight.

Asymmetry

The asymmetry of many parts of the human body including the brain, has been carefully investigated and the question arose as to the symmetry of the forebrain in the dog. The olfactory bulbs were removed first and then, following the usual method of dissection outlined above, the hemispheres and diencephalon were carefully divided into two halves. The incision was carefully made from above and checked as it came through to the ventral surface of the brain and in all cases it appeared to be in the midplane as nearly as could be determined. This was done for 84 male dogs and 101 females. Each half of the forebrain, without the olfactory bulbs, was carefully weighed in a glass stoppered weighing bottle and the weight of each half recorded. For these specimens, the sum of the two halves was taken as the weight of the hemispheres and diencephalon.

Of the 84 male dogs, 46 or 54.76 per cent had the left half of the forebrain heavier, and for the females, 60 or 59.41 per cent were likewise heavier on the left side. Both of these percentags were so close to 50 per cent that it was decided to get the differences in the average weights of the two sides, and the average weight of the left half of the forebrain for the males was found to be 31.648 grams and for the right half, 31.632 grams, or the average weight of the left half is greater by 0.05 per cent. The similar averages for the females are, 29.87 grams for the left halves and 29.78 grams for the right halves. This means that in the female dogs the left half averages greater than the right by 0.30 per cent.

With these very slight differences in the average weights and with the frequency of a heavier left half in 55 and 59 per cent of the cases, it seems that we are justified in saying that there is no significant variation between the left and the right sides in the forebrain of the dog. Coghill ('36) finds a greater asymmetry of the ganglia in the young of the Amphibia. There seems to be no general agreement with regard to the human brain, for Andreassi ('33) finds the left hemisphere greater by 0.17 per cent in men and by 2.81 per cent in

women. Higeta ('40) thinks that there is a greater difference in symmetry in the fetus than in the adult human brain and he lists five workers who find the left side greater and two who report a greater weight on the right side.

Correlations

The 10 parts of the central nervous system correlated with body weight, body length and also with brain weight are given in Table 3. The correlations between body weight and body length are given at the end of Panel *A*. All of the correlations are positive and all but one of the correlations in this table are above 0.5 per cent, and the aver-

TABLE 3

Correlations, Central Nervous System of the Dog

	162 male dogs	159 female dogs
A. *Correlations with body weight*		
Brain	+ 0.757 ± 0.023	+ 0.722 ± 0.026
Olfactory bulbs	+ 0.617 ± 0.033	+ 0.698 ± 0.027
Hemispheres and diencephalon	+ 0.746 ± 0.023	+ 0.688 ± 0.028
Proscencephalon	+ 0.731 ± 0.025	+ 0.697 ± 0.027
Mesencephalon	+ 0.542 ± 0.037	+ 0.678 ± 0.029
Cerebellum	+ 0.683 ± 0.028	+ 0.642 ± 0.031
Medulla	+ 0.795 ± 0.020	+ 0.772 ± 0.022
Rhombencephalon	+ 0.764 ± 0.022	+ 0.685 ± 0.028
Cord weight	+ 0.879 ± 0.018	+ 0.846 ± 0.015
Cord length	+ 0.435 ± 0.044	+ 0.940 ± 0.0064
Body length	+ 0.868 ± 0.013	+ 0.908 ± 0.0094
B. *Correlations with body length*		
Brain	+ 0.728 ± 0.025	+ 0.761 ± 0.023
Olfactory bulbs	+ 0.642 ± 0.031	+ 0.713 ± 0.026
Hemispheres and diencephalon	+ 0.704 ± 0.027	+ 0.714 ± 0.026
Proscencephalon	+ 0.714 ± 0.026	+ 0.735 ± 0.025
Mesencephalon	+ 0.538 ± 0.038	+ 0.723 ± 0.026
Cerebellum	+ 0.658 ± 0.030	+ 0.691 ± 0.028
Medulla	+ 0.740 ± 0.024	+ 0.775 ± 0.021
Rhombencephalon	+ 0.734 ± 0.024	+ 0.769 ± 0.022
Cord weight	+ 0.829 ± 0.017	+ 0.881 ± 0.012
Cord length	+ 0.875 ± 0.013	+ 0.981 ± 0.0021
C. *Correlations with brain weight*		
Olfactory bulbs	+ 0.750 ± 0.023	+ 0.765 ± 0.022
Hemispheres and diencephalon	+ 0.978 ± 0.0023	+ 0.991 ± 0.00098
Proscencephalon	+ 0.987 ± 0.0013	+ 0.994 ± 0.00069
Mesencephalon	+ 0.606 ± 0.034	+ 0.748 ± 0.024
Cerebellum	+ 0.833 ± 0.016	+ 0.807 ± 0.019
Medulla	+ 0.809 ± 0.018	+ 0.751 ± 0.023
Rhombencephalon	+ 0.869 ± 0.013	+ 0.805 ± 0.019
Cord weight	+ 0.777 ± 0.021	+ 0.744 ± 0.024
Cord length	+ 0.692 ± 0.028	+ 0.808 ± 0.019

age of the correlations in each of the three panels is greater in the females. The averages are greater in both sexes for the correlations with the body length and, as would be expected, still greater with the brain weight.

The length and the weight of the cord and the medulla form the three highest correlations with body weight and body length for both sexes, except for the cord length and body weight which is the lowest correlation in the males in Panel *A*. With this exception of cord length and body weight the order of the correlations is the same for the males, for both body weight and body length, or these correlations in decreasing order are: cord, medulla, rhombencephalon, total brain weight, prosencephalon, cerebellum, olfactory lobes, and mesencephalon. The high correlations of the hindbrain and the body dimensions are to be expected, but the cerebellum should be better correlated than it is if the cerebellum and the musculature, which forms a very large part of the total body weight, are as well related quantitatively as they are physiologically. The mesencephalon is largely a connecting pathway between the forebrain and the hindbrain and as shown in the earlier paper (Latimer, '24) it forms a much smaller part relatively, in the dog brain than it does in the anuran brain. The prosencephalon is sixth in order of the correlations in all four groups, males and females in Panels *A* and *B*.

Some of the correlations with the entire brain weight (Panel *C*) are high. The two highest, the prosencephalon and the hemispheres and diencephalon are very high but it must be remembered that they form approximately 83 and 81 per cent respectively of the total brain weight. If the order of decreasing correlations is checked with the percentage weights in Panel *C* of Table 1 it will be evident that these correlations are very nearly in the same order as the percentage weights of the total brain weight, or the relatively larger parts of the brain have the higher correlations with the total brain weight.

In general, the parts of the nervous system having the more primitive or fundamental relationships with the mass of the body, such as the cord and the medulla are better correlated with body weight and body length and the parts containing the higher associative centers are less well correlated with body size. Whether there is any correlation between the relative size of the prosencephalon and the "intelligence" of the dog we have no evidence, but the relative proportions of the brain do change without doubt from lower types of vertebrates to the more highly organized and in this larger sense there is a quan-

titative relationship between "intelligence" and relative proportions of the divisions of the brain.

TABLE 4

CORRELATIONS OF THE PARTS OF THE CENTRAL NERVOUS SYSTEM

	162 male dogs	159 female dogs
Olfactory bulbs and:		
Hemispheres and diencephalon	+ 0.669 ± 0.029	+ 0.731 ± 0.025
Prosencephalon	+ 0.708 ± 0.026	+ 0.746 ± 0.024
Mesencephalon	+ 0.427 ± 0.043	+ 0.609 ± 0.034
Cerebellum	+ 0.648 ± 0.031	+ 0.698 ± 0.027
Medulla	+ 0.652 ± 0.030	+ 0.693 ± 0.027
Rhombencephalon	+ 0.678 ± 0.029	+ 0.702 ± 0.027
Cord weight	+ 0.671 ± 0.029	+ 0.711 ± 0.027
Cord length	+ 0.642 ± 0.032	+ 0.785 ± 0.021
Hemispheres and diencephalon and:		
Prosencephalon	+ 0.988 ± 0.0013	+ 0.996 ± 0.00048
Mesencephalon	+ 0.578 ± 0.035	+ 0.725 ± 0.025
Cerebellum	+ 0.779 ± 0.021	+ 0.751 ± 0.023
Medulla	+ 0.766 ± 0.022	+ 0.689 ± 0.028
Rhombencephalon	+ 0.816 ± 0.018	+ 0.745 ± 0.024
Cord weight	+ 0.741 ± 0.024	+ 0.686 ± 0.028
Cord length	+ 0.661 ± 0.030	+ 0.781 ± 0.021
Prosencephalon and:		
Mesencephalon	+ 0.576 ± 0.035	+ 0.729 ± 0.025
Cerebellum	+ 0.788 ± 0.020	+ 0.769 ± 0.022
Medulla	+ 0.767 ± 0.022	+ 0.697 ± 0.028
Rhombencephalon	+ 0.809 ± 0.018	+ 0.752 ± 0.023
Cord weight	+ 0.728 ± 0.025	+ 0.715 ± 0.026
Cord length	+ 0.666 ± 0.030	+ 0.784 ± 0.021
Mesencephalon and:		
Cerebellum	+ 0.534 ± 0.038	+ 0.685 ± 0.028
Medulla	+ 0.668 ± 0.029	+ 0.872 ± 0.013
Rhombencephalon	+ 0.611 ± 0.033	+ 0.786 ± 0.020
Cord weight	+ 0.645 ± 0.031	+ 0.853 ± 0.015
Cord length	+ 0.543 ± 0.038	+ 0.738 ± 0.025
Cerebellum and:		
Medulla	+ 0.763 ± 0.022	+ 0.765 ± 0.022
Rhombencephalon	+ 0.962 ± 0.0039	+ 0.914 ± 0.0088
Cord weight	+ 0.725 ± 0.025	+ 0.775 ± 0.021
Cord length	+ 0.661 ± 0.031	+ 0.724 ± 0.026
Medulla and:		
Rhombencephalon	+ 0.889 ± 0.011	+ 0.845 ± 0.015
Cord weight	+ 0.906 ± 0.0095	+ 0.898 ± 0.010
Cord length	+ 0.745 ± 0.024	+ 0.801 ± 0.020
Rhombencephalon and:		
Cord weight	+ 0.852 ± 0.015	+ 0.808 ± 0.019
Cord length	+ 0.738 ± 0.025	+ 0.773 ± 0.022
Cord weight and:		
Cord length	+ 0.890 ± 0.011	+ 0.937 ± 0.0067

The correlations between each of the seven parts of the brain and the weight and length of the cord and the other eight divisions are shown in Table 4. All of these are positive and all but one are significant. All of the female correlations are 0.6 or above. In the males, there is one correlation of 0.4 (olf. bulbs and midbrain) and four between 0.5 and 0.6 and these are correlations of the mesencephalon with the prosencephalon, hemispheres and diencephalon, cerebellum and cord length. The mesencephalon seems to have the lowest correlations with the other parts of the brain and spinal cord. Of the 36 pairs of correlations, 12 are greater in the males and 24, in the females, or in general the parts of the central nervous system are better correlated in the female dogs. The parts which are better correlated in the males than in the females are the rhombencephalon and its two subdivisions correlated with the forebrain and the hemispheres and diencephalon, the rhombencephalon with its two divisions and the cord correlated with the medulla and the rhombencephalon. The highest correlations are between the forebrain and the hemispheres and diencephalon but this is to be expected when we correlate any structure with such a large subdivision of the total. The correlations between the rhombencephalon and its two divisions are also high but not as great as that between the forebrain and hemispheres. The correlations between the weight and length of the cord are high, especially in the female.

The correlations in this table as well as the constancy of the percentages of the parts of the brain with reference to the entire brain weight, show that the proportions of the brain are uniform and do not change with the size of the dog.

Summary

The averages, the coefficients of variation, and the significant ratios are given for the weights in grams of the male and the female brains, for the seven divisions of the brain, for the weight and length of the cord and for the body weight and body length. Similar statistical constants are given for the weights of these divisions of the central nervous system as percentages of the body weight and also for the seven divisions of the brain as percentages of the total brain weight.

The body weight is the most variable followed by the cord weight. The male brain is the least variable in weight but in the females there are three divisions of the brain which are less variable than the total brain weight. The coefficients of variation are much larger for these

weights when given as percentages of the body weight. The least variable are the seven parts of the brain given as percentages of the total brain weight. In all of these groups the females are generally slightly more variable than the males.

The weights in grams, with one exception, are significantly greater in the males but all of the percentages of the body weight are significantly greater in the females. There is no significant difference in the seven parts of the brain given as percentages of the total brain weight, but the forebrain in the females tends to be larger and the midbrain and hindbrain are slightly larger in the males.

Empirical formulae are given as a more accurate method of estimating the weights of any of the parts of the central nervous system from either the body weight or the body length for both the males and for the female dogs. The curves for the parts of the nervous system as percentages of the total body weight are all concave superiority, with the larger percentages in the smaller dogs. The parts of the brain as percentages of the total brain weight are all horizontal straight lines, which means that these percentages of the total brain weight are valid throughout the entire range of body weight for both the male and the female dogs.

The correlations of the parts of the system average a little higher with the body length than with the body weight and the correlations between the entire brain weight and the weight of its seven divisions are naturally still greater. All of the correlations are positive and all but one are significant. As a rule they are somewhat higher in the female dogs. The correlations between the various divisions of the nervous system are likewise positive and good correlations. The mesencephalon has the lowest correlations with the body weight, with the body length, with the total brain weight and with the other divisions of the system. The spinal cord and the medulla have the highest correlations with the body weight and the body length. The prosencephalon is sixth in order of correlations or a little below the average in each group of correlations. This seems to show that the cord and the medulla are more closely related to the mass of the body, and the forebrain seems to show some degree of independence of body size as far as its weight is concerned.

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THE ROLE OF THE AMINO ACIDS AND NUCLEIC ACID COMPONENTS IN DEVELOPMENTAL GROWTH: I. THE GROWTH OF AN OBELIA HYDRANTH

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The accumulation of the data on which this monograph is based was the work of many hands. These young people gave no thought to hours, and often worked from before sunrise to after sunset. They collected the little animals as faithfully in the cold drizzle of early spring and the hot gnat-infested summer afternoons as in the pleasant days. They waded shoulder-deep into the icy water of Massachusetts Bay, and swam beyond the breakers when storms came, so that the water used for our experiments would be clean and uncontaminated by flotsam and jetsam. In rain, wind, sand-storm, cold and heat, they brought thousands of gallons of clean sea water from the Bay up the hill to the laboratory. And no weather kept them from going down to the beach to scrub their white buckets clean with sand and water at the end of the day. Their names constitute an Honor Roll of workers in science. Their contribution was real. And I take great pleasure in thus acknowledging my debt and that of science to as fine a group of cheerful, loyal, interested, patient, honest assistants as could be found anywhere. Grateful acknowledgment is also made to those who painstakingly prepared the compounds used in this study, and gave them to me in a state of exquisite purity rarely equalled.

Laboratory Assistants

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B. General Preface

That which comes after ever conforms to that which has gone before.—Marcus Aurelius, Meditations, IV, 45.

1. *The Beginning*

The Lankenau Hospital Research Institute is the result of the vision and belief of Dr. Stanley P. Reimann: the belief that the problems of disease should be approached from the broad base of biologic fundamentals: the belief that the approach should be made in research institutes which are integral parts of general hospitals in order that the workers in the basic sciences should be enabled to obtain first-hand acquaintance with these problems. These beliefs were transmitted to Dr. Harvey Shoemaker and Dr. Henry Page, and through them to Mr. Rodman Wanamaker. Mr. Wanamaker thereupon presented The Lankenau Hospital with a building and equipment to be used as a research institute. In so doing he expressed the wish that the work of The Institute should be aimed at an understanding of cancer. His only proviso was that the line of approach should be one which was not being followed by other laboratories at that time. It was therefore proposed that the work of The Institute should center around the chemistry of growth, since cancer is a growth, and since the chemistry of growth was and still is largely terra incognita. This idea was accepted and work began in September, 1927.

Attention was first given to cell increase in number since this growth activity is a major contributor to tumor tissue increase. This work uncovered a naturally occurring chemical group of significance to proliferation. While these studies were in progress we were exploring in our mind the components of the broad problem of the chemistry of growth in order to determine more precisely the path of investigation we would choose to follow. The fact that the chemical group which had been found significant to cell increase in number was found specific to this growth expression and not directly concerned with cell growth in size; the fact that the group is a naturally occurring group; and the fact that the group or its precursors are naturally occurring constituents of living tissue wherever found, served to crystallize ideas into decision. This was, to begin finding the answer to

the question; What is the rôle of the naturally occurring tissue constituents of general distribution in the basic expressions of growth activity?

2. *The Point of View*

The phrasing of the question shows that interest is in fundamental rather than derivative aspects, since it proposes a tracing of relationship between a basic aspect of existence and a basic expression of living, i.e., between common substance and common activity.

It is only by making such attempts at tracing the integration of the basic phenomena of living that we can arrive at an integration of our knowledge thereon. The need for such integration need not be dwelt on here. But it is not amiss to point out that the mere collection and reporting of data, even though this be done by the 'scientific method' does not comprehend the 'meaning' or intension of science. For science is the data of the *relations* between things; between states; and between events; and the *relations* between things, states, and events.

The collecting of data as such may be as amusing as the collecting of stamps into books or of bits of string into balls. But it is as useless unless the relations are seen. Knowledge comes from data; understanding from relations between data. Of what use is knowledge without understanding?

Now all living things have a reaction basis through the exercise of which the basic phenomena that characterize the state of being alive are expressed. The common substance of living, of which growth is a common expression, is obviously comprised in a common reaction basis of living. This common substance is protein, cytoplasmic and nuclear. That is to say; the one substance common to all living things which provides the basic reaction basis common to all living things is protein, and it is through this protein-constituted reaction basis that growth comes to expression.

Although protein is the chief common constituent of living it in turn is built up of simpler compounds. For cytoplasmic protein these are the amino acids. For nuclear protein these are the amino acids plus the purine and pyrimidine components of the nucleic acid portion of nucleo-protein. These then are the basic naturally occurring tissue constituents of general distribution on which interest is focused.

It is legitimate to ask why interest is not focused on protein as such instead of on its components since it is the chief common sub-

stance of living. The reason is simple. Investigations into the nutritive value of the proteins carried out for now these many years by many workers in many laboratories have shown that the essentiality of any given protein in the diet for growth is not determined by protein as protein but by the presence in the protein of some particular amino acid or acids in sufficient amount. This being so the question to be answered now becomes: What is the rôle of the amino acids and nucleic acid components in the basic expressions of growth activity?

3. *The Nature of Growth*

Just as the 'substance' factor of the primary question was necessarily broken down into its components so is it necessary to break down the 'activity' factor into its components. It is held that growth is not single but multiple; the combined expression of incremental and developmental determinants, participants, and processes (47).

Any one who has observed a plant, an animal, a child, or a population grow from its beginning to maturity knows that this is accompanied and accomplished by something more than mere increase in mass and number. He knows that there is change in the proportionate representation of the parts in the whole. He knows there is specialization of the units which go into the make-up of the parts, whether this be of cells or parts of cells. And he knows there is segregation of like with like, whether it be of cells or of material within cells. That this point of view is acceptable is evidenced from the fact that the able biologist who devised the definition of growth for the 1939 edition of the Merriam-Webster New International Dictionary of The English Language caused to have printed therein that growth is "The progressive development of an organism or member from its earliest stages." Furthermore development as well as increase in size and number is included in the specification of the term. Any one who persists in and insists on restricting the meaning of growth to size and number increase would seem to convict himself of stubborn denial of fact. There are none so blind as those who will not see.

Returning to the concept that growth is the combined expression of incremental and developmental determinants, participants, and processes, there is here no need to consider the first two aspects. It has already been pointed out that the amino acids and nucleic acid components are the participants of present interest since they constitute in large part the substance of living, and it has also been

pointed out that the present inquiry is aimed at finding out in what manner they also may be determinants. Interest now then is focused on the processes of growth. These may be taken to be comprised under five headings.

The first is Anabolism. This deals with the processes concerned with increase in substance. The second is Initiation. This deals with the processes which are preparatory of new growth production. The third is Proliferation. This deals with the processes concerned in cell increase in number. The fourth is Differentiation. This deals with the processes concerned in cell specialization. The fifth and last is Organization. This deals with processes concerned in cell segregation.

These then are the basic components and aspects of growth and growth expression. They are the ones which are common to all living things at some time or another of their existence. The primary question may now be stated as follows: What part do the amino acids and nucleic acid components play in determining the expression of the basic aspects of growth as comprised in anabolism, initiation, proliferation, differentiation, and organization?

To finding an answer to this question has been my pleasant task for the past 13 years. This monograph is a coördinated report of the results of the inquiry. The results are by no means to be taken as giving the final answer. They provide, as it was hoped they would, a broad and solid foundation. A foundation of point of view, method of approach, knowledge, and understanding. But only a foundation. In order that the potentialities of this foundation may be realized much further investigation is needed. This is left for those who come after.

C. The Growth of an Obelia Hydranth

Man is certainly stark mad: he cannot make a worm, and yet he will be making gods by the dozen.—Montaigne, Book ii, Chapter xii.

1. *Introduction*

Before undertaking the research outlined in the General Preface it would seem good judgment to give thought to the choice of organism to be used as test material rather than to haphazardly take any which happens to be handy or of common use.

This is primarily not a study of an organism as organism; but of the components of a general property of all organisms. Because of

this it is desirable to use an organism in which these components are separable with reasonable distinctness.

The growth studies hitherto carried on suggest there is little inclination to consider growth as a coördinated expression of several activities, at least insofar as the experimental approach is concerned. This has given rise to the custom of designating the particular activity being studied as growth. In reality it is usually but one of the several aspects of growth. From this has come confusion regarding the meaning or intension of the term 'growth.' This in turn has tended not only to retard progress in understanding; but also to retard integration.

Clarity certainly comes from precision of statement. It would seem better to state that a study is a record of cell multiplication or body weight increase than to state it is a study of bacterial or body growth. Particularly when all that has been recorded is increase in cell number or increase in mass of the organism as a whole. And so on accordingly.

This tendency to lump everything under the one term growth has sometimes resulted in failure to utilize the opportunities offered by the test material to full advantage. It has also resulted in the use of organisms which are not conveniently suitable for a study of growth as the coördinated expression of basic activities.

A somewhat condensed account of our concept of the nature of growth with illustrations from the literature will serve to illuminate and establish the thesis.

2. *Initiation*

The substance of living comes from the lifeless. The plant makes its substance from the carbon dioxide and nitrogen of the air, and the inorganic water, sulfur, phosphorus and other significant elements of the earth. The animal derives its substance from the plant. As far as is known today the amino acids of which living animal substance is composed are as lifeless before they become incorporated as living as are the chemical elements from which they are made.

The transformation of the lifeless into the living is the becoming of growth. Attributes which are not exhibited in the lifeless state come to expression when this transformation occurs. What this transformation is, or what is the endowment of the chemical elements and already living substance which brings about the transformation is not yet envisaged or known. That unknown something called life shapes the course of things to come and the chemical elements and

their properties are merely the materials used for the shaping. All that is known is that there is chemical change and reconstitution. Perhaps a key to this most intriguing problem of all biology may be found in the crystalline viruses of Stanley (104).

It will be more productive of understanding of growth to consider the first expressions of this property of living as they exist to-day rather than to speculate on the processes which gave rise to growth in the beginning.

Chemical change and reconstitution is required for the initiation of growth regardless of the substrate from which it springs. This is the first step in new growth production from pre-existent living substance: living substance which is expressing the property of maintenance but not of growth. Examples of this phase of growth are given by the egg at the moment of fertilization whether this be accomplished by natural or artificial means (85, 93); by the dry and dormant seed when water is admitted; and by the aggregation and rearrangement of chromatin preparatory to nuclear division (84).

The processes of the initiation phase of growth are thus seen to be those which determine and accompany the shift in substrate activity from a state where maintenance is the chief expression to state where an activity specifically productive of a new structure is superimposed. They are the preparatory processes: the first changes in substrate which are growth changes.

Two propositions come out of this analysis. The first is that there are processes specific to initiation. That is to say, processes which are distinct and different from those determinative of the expression of any other growth activity or indeed of any other property or function whatsoever. This is not to be taken to imply that they exist isolated and alone like gas molecules in vacuum. Such would be ridiculous. They are related to and derived from antecedent processes, and they themselves are antecedents of subsequent processes.

The second proposition is that there are processes peculiar and specific to initiation which are common in kind and participants to all expressions of this growth activity wherever found. This concept derives from the fact that the activities of living, of which growth initiation is one, take place in a substrate which is common to all living things with respect to the nature of its constituents, viz., the amino acids and nucleic acid components. These activities derive from and are given expression by this common substrate.

The test materials cited in a preceding paragraph are valuable

adjuncts to the demonstration of other growth activities than initiation. The egg and the seed may be used for studies of the fact of mass increment; the fact of proliferation; the fact of cell specialization in cytogenesis; and the fact of cell segregation in histogenesis. And the dividing cell can and should be used for study of the intracellular expression of these basic activities. This last has yet to be done. The cell of course grows like the organism grows and in growing expresses the same basic activities within itself as the organism does. Indeed the growth of organism is but recapitulation of growth of cell.

Now in order to get an answer to the question: *Is a given amino acid of particular significance to any of the basic growth activities?* it is desirable to use an organism in which it is possible to follow, separate, and measure each activity as it comes to expression in the same living individual. Otherwise there is but little distinction in difference.

Obviously then these test materials and others like them are not very suitable for such a study for two reasons. In the first place they must be killed and sectioned before the progress of events can be measured and determined. This gives a time slice instead of a continuum for observation and study. Which means that the growth of an individual cannot be followed. In the second place the boundaries between the successive steps from beginning of growth to structural maturity can not be determined with even fair approximation. They show as stated above the fact of mass increment, initiation, proliferation, differentiation, and organization. But they do not allow separate measurement or even estimation thereof with reasonable accuracy. They allow measurement of growth but not of its components. It would seem as if other than those test materials would be more conveniently suitable for the present study.

3. *Proliferation*

Proliferation, the second step in developmental growth progression, is a common sequence of the primary initiation process. It is increase in cell number.

The cell is commonly thought of as a structural unit. Most cytologists would agree that the cell is much more than this. Yet the idea of the cell as a boxed-in affair persists. It tends to overshadow the fact that in multicellular organisms the cell is not an individual entity in which mysterious events occur in isolated fashion. It is hard to break away from traditional ways of looking at things. Yet this must be done if concepts are to grow with knowledge. Lag in integrating concepts

with knowledge is of course inevitable. But as far as the cell is concerned the time has come to discard the concept of the cell as a structural unit and to replace it with a concept more in accord with the facts.

The cell composed of nuclear material and its associated cytoplasm is not alone a structural unit: it is the center of a sphere of influence. There is substance continuity between cells by protoplasmic bridges and syncytia. The reactions of cytoplasm associated with one nucleus have their reverberations in the activities of the cytoplasm associated with contiguous nuclei. This mutality of influence is affected as much through the continuity of living protoplasm of one cell with another as it is by extracellular diffusion of intracellular reaction products. When one nucleus becomes two there is concomitant association of functionable cytoplasm with each. When in amoebae, the plasmodia, syncytial tissue and the like, there are two or more nuclei, then there are two or more centers of influence. These comprise two or more activity units, or two or more cells in the chemical and physiological sense. This holds even though there be no visible separation of cytoplasm by encircling boundary. An amoeba with two nuclei is a two-celled amoeba, and a multinucleate plasmodium is a multicellular plasmodium regardless of the absence of inter-cellular boundaries.

Proliferation is therefore taken to be increase in number of active units of living substance. This is the second phase of developmental growth expression.

Examples of proliferation activity are seen in the cell increase in number of tissue cultures (76) and cultures of unicellular organisms (114); the mitotic figures of killed and sectioned growing material such as root-tips (38) and regenerating (43) and embryonic structures; and the increase in cell number per unit area in sections of appropriately chosen tissues (48).

Now anyone who has seen the structural changes incident to cell division needs no further evidence that there are processes specific and peculiar to proliferation. For no other cell activity exhibits such phenomena. Obviously these are expressions of chemical reaction and physical change (24). It follows then that the chemical processes which produce these structural changes are themselves specific and peculiar to proliferation. These processes are the ones which determine and accompany the redistribution, resynthesis, rearrangement, and separation of the substance of one cell into two. They are distinct and different from all other growth processes and indeed from any other property and function whatsoever. They have their

own factors, determinants, and participants. They are, however, related to and derived from antecedent processes, and they themselves are antecedents of subsequent processs.

Back of the word "determine" as used in biological exposition there are two meanings and it would seem wise to clarify them here in order to avoid future misunderstanding and quibble.

In the preceding paragraph "determine" is used in the sense of—to bring about a certain result (by an irreplaceable thing, state, or event).

Thus proliferation is a determinant of growth because its contribution to this activity cannot be replaced. Redistribution and separation of cell substance are determinants of proliferation because they contribute to its expression and their contribution cannot be replaced by any other activity. And certain chemical groups and processses determine redistribution and separation because no others can bring about a like result.

Now that which determines what particular chemical groups and processes out of infinite possibilities shall be used for growth is another matter. And so is that which determines the nature of the redistribution and separation of cell substance which is peculiar to proliferation. And so is that which determines the part of proliferation in growth, and that growth shall produce the forms it does. Indeed, that which determines that growth itself shall be an aspect of living is likewise another matter.

In all these there is the idea that the stated phenomena are directed along a certain course to a certain end. Here then "determine" is used in the sense of—to fix the character or form of beforehand; to direct as to a certain end; to give a direction to or to fix the course or end of. This is the other meaning of the word as used in biological exposition.

What that something is which determines the course and end of growth is beyond present knowledge and understanding. For want of definition it is called "life".

To recapitulate: Growth is brought about as a result of (determined by) properties and processes inherent in and peculiar to the substances used for growth. The existence of growth as a basic aspect of living and the nature of its expression is fixed and directed (determined) by a function of living. The former is factorial determination, the later directive.

It is evident that in any given tissue where proliferation is taking

place there are processes specific and peculiar to this growth activity which are distinct from those used in the bringing to expression of any other growth activity. It is not so evident, however, that there are processes specific to proliferation which are common thereto when—and wherever it is expressed.

Objection to such a concept comes from the fact that there are several types of cell division. Thus some cells divide by karyokinesis and other amitotically. At least the intricate nuclear changes of mitosis are not immediately apparent in the latter. Others, such as yeast cells, multiply by budding; and in others, such as the blue-green algae, where there is nuclear material but no formed nucleus, division seems to be a simple cleavage.

This objection, however, is not a serious bar to acceptance of the major premise. Now all cells express the same basic growth activities, either in retrospect or in prospect. But the manner and result of their expression is affected by other factors. Thus the cell activities which characterize a species are determined by species-specific factors; and the cell activities which characterize a tissue are determind by histo-specific factors; and the cell activities which characterize an organ are determined by organo-specific factors.

The way in which a cell divides is therefore the result of the combined influence of these several factors. But cell increase in number is cell increase in number wherever it occurs. And whenever one cell becomes two by natural procedure the substance of the mother cell is redistributed, resynthesized, rearranged, and separated into two activity units or centers of influence. In other words the substance of any dividing cell undergoes certain basic transformations which are specific and peculiar to proliferation and which are common to this growth activity when and wherever it occurs regardless of the origin, structure, or mode of division of the cell.

It may be helpful to be a little more explicit about these transformations.

In order that cell substance may be separated by natural procedure so that two cells appear where there was but one before it is obvious that some preliminary degree of loosening of the chemical bonds which hold the cell components together as a singly functioning aggregate must be had. This transformation is one of preparation for division.

In order that even a modicum of homogeneity between daughter and mother cell may be had it is necessary that there be redistribution of the loosely bound substance of the parent cell as the beginnings of

the two centers of influence are in the making. This transformation is one of distribution of cell substance.

In order that the reaction basis peculiar to the non-dividing cell may be formed in both mother and daughter cell it is necessary that the loosely-bound redistributed cell substance undergo resynthesis into the characterizing aggregate. This transformation is one of resynthesis of cell substance.

In order that the redistributed and resynthesized cell substance may attain the structural and functional state characteristic of the cell type it is necessary that there be rearrangement thereof into the requisite segregations. This transformation is one of rearrangement of cell substance.

Finally there is the separation of daughter and mother cell into individual centers of spheres of influence by boundary or cell formation such as occurs in most cases, or as in special cases by an imperceptible association of cytoplasm with the nucleus.

Anyone who has thought at all about what underlies the structural changes in mitosis, or the multiplication of unicellular organisms, or the beautiful moving pictures of cells in division exhibited by Warren Lewis, knows that these transformations of cell substance are basic transformations and hence that they occur where and whenever cells increase in number by natural procedure. They know that cell division is not a succession of static frozen postures, but a continuous dynamic flow of chemical and physical change.

The analysis perforce is resolved into a transposing of morphologic aspect to chemical concept. And such is needed. For far too long has biological interest been focused on the morphology of cell proliferation and its genetic implications to the almost complete exclusion of interest in the chemical processes from which they spring. This of course has come from the fact that but few biologists have sufficient chemical knowledge and training to endow them with inclination or ability to think of proliferation or any other growth activity in terms of chemical change. True it is there have been isolated speculations in this direction but these have been more futile than fertile. The present exposition should make evident the usefulness of bringing into proper focus the chemical basis of growth in all its aspects.

Granted that the transformations described above are common to cell increase in number and that they are expressions of chemical change the question naturally arises as to the basis of the proposition

that each is everywhere subtended by the same chemical processes and participants.

The answer lies in the fact that they take place in, derive from, and are expressed by a substrate which is everywhere common with respect to its basic components, viz., the amino acids and nucleic acid components. In view of the fact that the same chemical groups and bonds are everywhere available for proliferation it would be strange indeed if the same chemical processes were not everywhere used for expression of the same proliferation activity.

To recapitulate: Proliferation is a growth activity which is common at one time or another to living substance wherever found. It has its own chemical determinants, participants, and reactions which are common to its expression wherever this is exhibited, and which are distinct from those which give expression to any other growth activity.

While this postulate is evidently valid without further exposition it will not be redundant to present some supportive data derived from direct experiment.

4. *The SH Postulate*

If the postulate is correct any basic determinant of proliferation must fulfill the following requirements. It must be a naturally occurring constituent of living substance. It must be actually or potentially present in adequate concentration wherever cells are dividing. Its effect on division must always be the same when proper concentrations are provided. This effect must be produced on all types of cells regardless of their origin, structure or mode of division. And it must have no direct effect on any other growth activity.

The SH or sulfhydryl group comes pretty close to satisfying these requirements.

Since the significance of the group to proliferation was established by work done at The Lankenau Institute, and since the several reports are scattered this is as good a place as any to bring the evidence together and tell the story of its unfolding.

As stated earlier our beginning interest was in proliferation. At the time work began there was a great to-do over the possible value of Lead (Pb) in the treatment of cancer. The idea was being emphasized by Blair Bell who claimed remedial effects from its use, and that these derived from inhibition of proliferation (2). While we were convinced from purely chemical and toxicological data that the metal would never be of any practical use in cancer therapy (which

conviction has long since been sustained though only after much needless expenditure of time and funds which might have been more profitably spent) we were interested in the possibility that Lead (Pb) might be a useful tool in opening up knowledge of the chemistry of cell division. Providing of course that Blair Bell's contention of its more or less specific action on dividing cells was correct. The matter deserved looking into.

One night, in September, 1927, to be exact, just to see what would happen, a couple of small crystals of Lead nitrate were thrown into a beaker in which the sprouting roots of some onions were growing in distilled water. The next morning to our astonishment and delight the tips of these roots were colored a brownish black for about a millimeter back from the root-cap. The rest of the roots were their usual pearly white against which the blackened tips stood out in sharp contrast (36). The discoloration was not a deposit on the surface of the tips but was a change within the substance of the roots themselves.

Now root growth in length is accomplished by cell increase in number and cell increase in length. And cell division is concentrated in the tip while cell increase in length (size) is the chief growth activity posterior thereto. This being so the simple experiment just described showed four things. It showed that the substance which reacted with the Lead (Pb) was of natural occurrence. That this was concentrated in the region of cell division. That it was specific and peculiar to that region. And that the region is chemically as well as developmentally distinct from the region of growth by cell elongation.

The experiment however did not show that proliferation was affected by Lead (Pb), or how; nor did it tell the nature of the substance reacting with the metal, or whether or not the substance was a determinant of proliferation activity or simply a product thereof. While the black color suggested that Lead sulfide had been formed, and evoked the memory that mercaptan formation is characteristic of the onion, it did not bring to mind any idea that sulfur in any combination is of any particular significance to proliferation. It merely indicated that the discoloration induced by Lead sulfide formation might interfere with observation, and that in order to avoid this in further experimentation it would be necessary to use other material than the onion with its special mercaptan metabolism.

The first step in quantitative analysis was the finding that the length growth of roots of sprouting corn and beans was retarded in balanced culture solutions containing Lead nitrate (34). The second

step was the finding that this came from retardation of cell increase in number and not of cell increase in size (36). There were fewer mitoses, both absolutely and relatively, in the Pb-treated roots, and the cells were no smaller. Evidently Lead (Pb) in the concentrations used here had a specific retarding effect on proliferation.

It should be emphasized that cell size as well as cell division should be measured whenever possible. Otherwise it is impossible to allocate the influence of any given procedure with precision. In working with plant material especially, such as roots, and coleoptiles, it is not enough to state that "growth" is affected. It is necessary to come as close as possible to finding out what phase of growth is affected, not only from the point of view of scientific exactness, but also from the point of view of preventing confusion in the minds of the unknowing and the unthinking. It is work, I admit, to make serial sections, count cells, and measure diameters in a sufficient number of specimens for valid estimation. And any picture of such sections has far less dramatic appeal than a photograph of a row of pretty coleoptiles bending before the auxin breeze. But science is science and the stopping at half-way measures when the material allows fuller analysis would seem to stamp the worker either as unknowing and incompetent or as somewhat deficient in scientific integrity.

Correlative evidence that Lead (Pb) inhibits cell division specifically was had in the finding in free-hand sections of living roots that the metal is concentrated in the nucleus rather than in the cytoplasm (35). Growth by increase in cell number centers in the nucleus; growth by increase in cell size is largely cytoplasmic.

This was supplemented by the finding that the areas where mitoses were most numerous in the normal root were just the areas where the greater number of Pb-fixed nuclei occurred in the Pb-treated specimens (71). And finally it was found that there was no difference in principle between plants and animals for Lead (Pb) was concentrated in the regions of greatest cell division activity in very young chick embryos as it was in the root-tips (74). The evidence is unequivocal that cell division is specifically inhibited by Lead (Pb).

During the study of the free-hand sections of living root-tips it was noticed that a colorless precipitate was always present in the meristematic region of those which came from Pb-containing cultures, while none such was in the controls. This precipitate was found only sparsely if at all in non-proliferating areas. This finding evoked the idea that the inhibitive action of Lead (Pb) on proliferation might

be due in some part to the precipitation out of some chemical compound essential to cell division.

Naturally the next step was to determine the chemical nature of this localized precipitate. Analyses were therefore made of it as found within the living cells of free-hand sections under the microscope. It was found that the precipitate was none of the possible inorganic combinations but was a combination between Lead (Pb) and some unoxidized sulfur compound analogous to, if not, glutathione (37).

Similar analyses were made of the brown-black product in the Pb-treated onion roots. This also was found to be a Lead-sulfur combination, viz., PbS. These findings pointed towards reduced sulfur as a significant association of proliferation.

The literature told that Gola as far back as 1902 had gotten a positive nitro-prusside test in root-tips (25), and that Hopkins in 1921 had attributed the reaction to the SH or sulfhydryl group of glutathione which he had just isolated and identified (79). The nitro-prusside test was therefore applied to our control and Pb-treated roots. It showed uniformly that sulfhydryl was concentrated in the meristematic region and essentially absent from non-proliferating areas posterior thereto (9). The positive reaction however was always very faint in the Pb-treated specimens, while in the controls it was vivid and strong. Thus it was shown that sulfydryl is a significant associate of proliferation.

Therefore, since Lead (Pb) inhibits cell division; since Lead (Pb) produced a precipitate in roots; since this precipitate was concentrated in regions of mitotic activity and essentially absent from non-proliferating regions; since the precipitate was a combination of Lead (Pb) with reduced sulfur (SH); since sulfhydryl was found concentrated in the meristematic region and essentially absent from non-proliferating regions; since the distribution of sulfhydryl and the Pb-sulfur compound was the same; and since the amount of sulfhydryl in the meristematic region of Pb-treated roots was much less than in the untreated, there was but one conclusion possible, and that was that Lead (Pb) is a retardant of cell division because it precipitates the sulfhydryl group out of action.

The natural corollary to this is that sulfhydryl is essential to proliferation. Interest was therefore transferred from Lead (Pb) as an inhibitant of proliferation to sulfhydryl as a possible specific determinant of this growth activity. Thus it is seen how one thing leads to another and scientific knowledge unfolds step by step.

The hypothesis was put to test.

Although the above experiments gave no indication that any group or compound other than sulfhydryl (SH) was implicated, it was deemed necessary to plan the experiments so they would show whether it was this group or some radicle to which it might be attached which was significant. So the first experiments were run with sulfhydryl attached to many different organic radicles, and controls were run, not only in plain culture solutions but also in solutions which contained the compounds used for the test in which SH had been replaced by an inert substitute such as hydrogen.

Roots of sprouting corn and beans were used.

The first step was to determine the effect of sulfhydryl on root growth in length. It was found that an increase was produced. This could have resulted either from cell increase in number or cell increase in length, or both. So the next step was to make counts of mitotic and resting cells of the same roots. This showed that the presence of sulfhydryl in the culture solutions in non-toxic concentration brought about an absolute and relative increase in the number of mitoses.

While this was sufficent evidence that SH acted to stimulate cell increase in number it did not eliminate the possibility that cell increase in size was also enhanced. It was necessary to examine this for two reasons: first, and most important, because of the need to see if sulfhydryl was specific to proliferation and not directly concerned in mass increment and second because of the claim that cell size is a primary determinant of division (9) and hence the possibility that the cells had been brought to earlier division because of mass increment.

Therefore as a final step in analysis of the growth reaction measurements were made of cell diameters. These showed that the cells of the SH-treated roots were smaller and shorter than those of the controls. This checked with studies made on other normal roots which showed pretty conclusively that the more rapid the division rate the smaller the cells (38). The conclusion was obvious that the greater root growth in length was due to stimulation of cell increase in number and not of cell increase in size, and that the influence of sulfhydryl was specific to proliferation.

The sum and substance of these studies then is that, for root-tips at least, sulfhydryl is a naturally occurring chemical stimulus essential to growth by increase in cell number (39, 40).

It should be noted in passing that since the reaction was produced by all the different SH-compounds regardless of the radicle to which

the group was attached, and since it was not produced by the same compounds without SH, the result must be attributed to sulfhydryl as such and not to the radicles.

While extension of this conclusion to all proliferation would be legitimate on the basis of previous considerations alone, substantiating experimental evidence is available. Thus sulfhydryl stimulates normal (40), pathological (40, 42), reproductive (100), embryonic (29), regenerative (68), repair (72), and replacement (95, 41) proliferation. Immature cells of different kinds of tissues are stimulated to more rapid proliferation, such as ecto-, meso-, and entodermal derivatives (43), epithelium (68, 41), connective tissue (41), muscle (68) and nerve cells (44). And the reaction occurs in all the different species so far tested—from the lowest to the highest, viz., blue-green algae (75), corn and beans (40), amoebae (108), paramecium (40), hydroids (45), fruit-flies (112), worms (43, 44), snails (29), hermit-crabs (68), fish (29), tad-poles (105), rodents (72), and man (96).

The data show that sulfhydryl fulfills the requirements demanded of a specific determinant of proliferation. It is a generally distributed naturally occurring constituent of living substance (79, 40, 82, 90). It is actually or potentially present in adequate concentration wherever cells are dividing (79, 40, 82, 90, 107, 117, 5, 20, 11, 64, 10). Its effect on division is always the same in kind when proper concentrations are provided (40, 46, 100, 29, 68, 72, 95, 41, 44, 75, 108, 45, 112, 105, 110, 94). This effect is produced on all types of cells regardless of origin, structure, or mode of division 40, 46, 100, 29, 68, 72, 95, 41, 44, 75, 108, 45, 112, 105, 110, 94). And it has no direct effect on any other growth activity (40, 68, 41, 65).

Further citation is unnecessary. While all possible trials have not been made, the gamut of species, cell type, and mode of division has been sufficiently covered to justify the assertion that this particular chemical group is of specific and universal significance to proliferation. This single fact is all that is sufficient and necessary to validate the concept that there are processes specific and peculiar to proliferation whenever, wherever, and however it may be expressed. Which is what we set out to demonstrate some pages back.

The foregoing is but a scant sample of the test materials which have been used for the study of proliferation. It is probable that more different kinds of cells and species have been employed for study of cell increase in number than for any other single growth activity not even excepting mass or size increment. This is not only because the evolu-

tions of mitosis are of real interest, but also because in practically every case cell multiplication is the only growth activity which can be recognized in clean-cut isolation and separately recorded with quantitative accuracy. Because of this, and for reasons given under Initiation the test materials cited are not conveniently suitable, with one exception, for exploration of the part a given compound may play in developmental growth. This exception is the colonial-living form of the marine hydroid Obelia. Its suitability will be demonstrated.

5. *Differentiation*

The processes of proliferation are the prelude to differentiation—the next phase of developmental growth progression. Differentiation is cell specialization—specialization in composition, structure and function. This specialization is expressed by cell substance. Cell substance is chemical in nature. Therefore differentiation is chemical specialization.

Examples of differentiation are innumerable. They can however be grouped under six headings which represent some of the chief occasions in which the activity is a participant in the growth picture. They are as follows:

(*a*) In unicellular organisms such as paramecium (40), blue-green algae (75), and the like, differentiation is expressed by a post-divisional intracellular reconstitution of cell substance to the status of the parent cell.

(*b*) In multicellular organisms it is expressed in the derivation of different cell types from proliferation matrices in early development such as occurs in the production in roots of covering, supporting, and translocation elements from the tip (101), and in eggs of crepidula (13) and other species (84) of ecto-, meso-, and ento-derm and their derivatives.

(*c*) And it is expressed in the development of these cells towards maturity in the increase in compositional, structural, and functional specialization such as is seen in muscle, bone, and nerve cells of embryonic and regenerating structures (68).

(*d*) Differentiation also participates in the production of cells for special maintenance function such as surface protection, reproduction, oxygen transport, and the like. Thus it is expressed in the transformation of basal epithelial cells into the keratinized cells of the epidermis; it is expressed in the maturing of spermatids into sperma-

tozoa; and it is expressed in the development of white and red blood cells from the leucoblasts and premyelocytes of the bone-marrow.

(*e*) Differentiation is expressed in repair growth of which regeneration and wound healing are typical. In both there is antecedent cell damage from mechanical injury. In both there is probably liberation of sulfyhdryl from the damaged cells and resultant stimulation of proliferation (40). In regeneration differentiation is expressed in the reconstitution (12) of the cells which go on to reproduce the structure which was lost. Such is seen in Clymenella (43) and Pagurus (68). In wound healing differentiation is expressed in like changes in the cells which here proceed to repair and cicatrize the damaged area. Such is seen in rat (72) and man (105).

(*f*) Finally differentiation is expressed in pathological growth such as cancer. The change from body cell to cancer cell is one of specialization. But cell specialization is differentiation. Therefore the change from body cell to cancer cell is an expression of differentiation. The fact that l-Proline, which has been found to forward differentiation expression in Obelia (67) and other organisms, also brings about an increase in the number of multiple tumors in mice (59) is experimental support for this concept. It is true that the appearance of greater numbers of tumors can result from stimulation of proliferation of scattered tumor cells, as well as from a stimulation of the change from body cell to cancer cell. But since l-Proline does not act to stimulate proliferation, indeed quite the contrary, but does act to stimulate differentiation, the conclusion is justified that it is differentiation and not proliferation which is affected in the case of mouse tumor production.

Differentiation is also expressed in the cancer cells after their derivation from body cells. The products of their proliferation show a tendency to specialize in the direction of that exhibited by the cells of their tissue of origin and occurrence. The degree of specialization ranges from a mere hint to a quite satisfactory accomplishment. Thus in some breast tumors of mice acinar cells of a very low grade of specialization may predominate; in others the majority may be well developed; while in others many different grades may occur (61).

These then are the six chief occasions of differentiation participation in the growth picture, viz., the reconstitution of daughter to the status of parent cells in unicellular organisms; the derivation of new cell types from proliferation matrices in embryonic multicellular organisms; the progressive specialization of cells in composition, structure,

and function; the maturing of cells of special non-developmental function to replace those lost in the exercise of that function; the reconstitution of cells after injury for regeneration and repair; and the transformation of body cells into cancer cells.

These examples show that growth by differentiation—like all other—is a becoming—not a sudden acquisition. It begins with the redistribution and resynthesis of division. It is completed with the attainment of that structural maturity consonant with optimum functional effectiveness—when the chemical processes are no longer greatly concerned with producing a cell of specific chemical composition; but with expressing the function which that composition affords and with the maintenance of that composition under the wear and tear of functional expression.

It is clear from this that there are processes which are specific and peculiar to differentiation—processes which are distinct from those of any other growth activity or indeed of any other functional activity whatsoever. Obviously these derive in part from antecedent processes of proliferation and are themselves antecedents in part of subsequent processes of organization.

In view of the fact that differentiation is the primary growth activity through which the distinctions between cells in composition, structure, and function are brought to expression, and the fact there are so many diverse products of this expression, it is a little more difficult to see that there are processes specific and peculiar to the activity which also are common thereto wherever it may be expressed. The concept can be illuminated by consideration of the processes and factors involved therein.

It is convenient to assume two sets of general processes for differentiation; the enabling and the expressing. The first may be taken to be comprised in the chemical changes of cell substance which endow daughter cells with the ability to specialize. The second may be taken to be comprised in the changes which direct and accomplish the specialization.

Each set of processes has its own determinants, participants, and reactions. The enabling factors determine that the cell shall specialize. The expressing factors determine what that specialization shall be.

Consider first the basic directive factors which underlie cell specialization. They are as manifold as the cells they give rise to. They are essentially genetic in character. They may be grouped under three headings.

There are the species-specific factors. These are phylo-genetic in character. They determine the species individuality in cell composition. Through them the cells of oaks and oats are set as oak and oat cells, and the cells of snakes and snails are set as snake and snail cells. And so on similarly.

Then there are the organo-specific factors. These are paragenetic in origin. They determine the parallelism in cell composition and function of like organs of different individuals and species. Through them the roots of both corn and bean are set to have cells with root-hairs, and the muscle cells of both cats and mice to have fibrils. And so on similarly.

And finally there are histo-specific factors. These are ontogenetic in origin. They determine for a given organism the tissue specialization of cells in structure, function, and composition. Through them leaf cells are set to contain chlorophyll, and stem cells to contain starch; while nerve cells are set to have Nissl bodies, and liver cells to have canaliculi. And so on similarly.

There can be no doubt that the infinitely diverse appearances of cells, their developmental expression, and their true breeding are the result of genetically determined directive factors. Else there would be chaos instead of order in nature. The existence of such factors has been experimentally demonstrated.

Thus when immature cells from the site of origin of a limb in embyronic development have been transplanted to another site in the same organism, and have, if they survived, seemingly given rise to a limb appropriate to the site of origin rather than to the site of implantation; this may be taken as evidence there were present in the transplants directive factors for differentiation of their cells into limb cells. Factors which were specific to limb cell production. Factors which were histo-specific in nature and onto-genetic in origin.

And when cells of meagre developmental maturity such as those of the blastomere, have been similarly transplanted from one region to another, and have, if they survived, seemingly given rise to a growth appropriate to site of implantation rather than to the site of origin, then this may be taken as evidence there were present in the cells, normal to the site of implantation, directive factors for differentiation of the cells of the transplant into cells appropriate to the new site (102).

Such factors have been called inductors, evocators, and organizors with the implication the function is solely concerned in organization. This seems unwarranted. Obviously differentiation of cell substance

precedes organization of the cells into functionable aggregates. And in the cited experiments, particularly those of blastomere transplantation, the cells of the explant must have taken on the specialized attributes of the cells of the region of implantation before they could have gone on to produce the organized structure appropriate to the region. This is not to deny the existence of organizors in developmental growth. It is merely to point out that the results noted above should be attributed as much to directive factors for differentiation, or differentiators, if a word must be coined, as to the presence of factors specifically concerned in organization.

Certain questions merit attention, viz.:

(*a*) *What is the action of the differentiator?* The differentiator sets the cell in the path of its specialization. This is shown by the fact that transplants of a limb bud anlage may go on to produce a limb appropriate to the site of origin rather than to the site of implantation.

(*b*) *Where is the action of the differentiator exerted?* The differentiator exerts its action on the redistribution and resynthesis of cell substance which occurs during division, and on the pre- and post-divisional redistribution and resynthesis which continuously accompanies cell activity (106). It is logical to assume that the beginnings of cell specialization occur as stated since it is here that the bonds for new direction of cell substance arrangement are presumably freed. Supporting the assumption is the fact that immature cells which ordinarily do not go on to further differentiation to any marked extent, may be brought to rapid differentiation through being stimulated to proliferate by SH (72, 41).

As to action in pre-divisional rearrangements it is pretty certain that the differentiators which set body cells to become cancer cells act *before* the cells divide to produce the malignant growth, and that the subsequent exacerbation of proliferation activity is the result, not the cause, of the acquisition of malignant property. There is no evidence that proliferation per se is responsible for the cancer cell (46). There are many cases of record where proliferation has been stimulated without subsequent cancer production (41, 98). Apparently the neoplastic diathesis of the cell is acquired before the cells divide to produce the cancer.

If the hypothesis that cells which are to regenerate a part lost by injury undergo reconstitution before they go on to reproduce the part is correct (12), then here too it is possible that the differentiator exerts its action on the pre- as well as the divisional changes.

And finally it is conceivable that the post-divisional state of flux which immediately succeeds division and which precedes the attainment by the cell of its characterizing attributes is also hospitable to the action of the differentiator, since here too bonds are presumably still open to directive rearrangement into new patterns.

(*e*) *When does the differentiator exert its action?* The differentiator exerts its action before the path of specialization of the cells has been set. This is shown by the fact that blastomere cells may take any of several paths of specialization depending on whether they are left *in situ* or transplanted to other regions. For this is evidence that the differentiator which determines their path of specialization must exert its action while their substance is still in an indeterminate state insofar as developmental progression is concerned.

(*d*) *How does the differentiator exert its action?* The differentiator exerts its action by inducing a rearrangement of cell substance through neo- and re-constitution. This is shown by the changes in appearances of cells as a lineage is being established by successive divisions in embryonic development (13); both before the histo- and organo-definitive characteristics are foreshadowed or fixed, and at the moment of fixation. Such as can be followed as the immature muscle cell is progressively derived from the mesoderm. Furthermore, the very fact that the muscle cell differs so strikingly from its cell of origin in composition and structure is evidence enough that neo- and re-constitution of cell substance is a *sine qua non* of specialization.

(*e*) *What is the nature of the differentiation?* The differentiator is chemical in nature. Although naturally occurring differentiators have yet to be surely isolated, purified, and identified there can be no reasonable doubt but that they are chemical compounds or groups of specific composition and arrangement. Evidence for this is had in the fact that the specialization of body cell to cancer cell is evoked by compounds of known and definite composition (14), and the fact these are potentially related to naturally occurring compounds through the common possession of certain characterizing configurations.

The foregoing may be summarized as follows: The directive factors for differentiation expression are genetic in character and derivation. Through them is established the pattern which the cell is to follow in the completion of its developmental growth. The development of this pattern through successive divisions is accomplished largely by rearrangement and selective recombination of cell constituents.

It is clear from the diversity of end-products that while there are

factors specific and peculiar to differentiation there is hardly liable to be found among the directive factors as such any which are common to every expression of this growth activity.

But this is not all there is to differentiation. The mere setting of a cell on its path of specialization, and the fixation of the pattern which this is to be, does not mean that the cell by itself is going on to the particular compositional and structural differentiation which is characteristic of its maturity. There is a gap between the directing of a cell towards a certain end and the reaching that end. The closure of that gap is the business of certain processes which may be called the effective processes for cell maturity. These processes are comprised in selective assimilation.

Assimilation is here taken to be the incorporation into cell substance of additional building material from which comes cell growth in size; and selective assimilation is the addition to cell substance of just those compounds and groups which are specific to the function and structure of the cell as set by the genetic composition during developmental growth. It derives from the presumed ability of the cell to pick and build into itself from the heterogenous assortment presented in the milieu those compounds in kind and number which are requisite to its specialization. It is clearly distinct from the intracellular neo- and re-constitution of already present cell substance which sets the pattern of differentiation. It is expressed when cells increase in size without change in pattern save in the direction of increasing definition.

Briefly then the expressing processes for differentiation may be taken to be composed of intracellular neo- and re-constitution of cell substance, and selective assimilation of building materials from the milieu.

As the cell type becomes more and more set apart from its precursor in successive divisions through augmentation of its compositional, structural, and functional definition—such as occurs in the derivation of nerve cells from ectoderm and of muscle and bone cells from mesoderm—the relative contribution of the two sets of processes progressively changes. Thus the part played by intra-cellular neo- and re-constitution is greatest at the beginning and decreases with each successive division, while selective assimilation is least in the beginning and increases as divisions become less frequent. Indeed it may be held that differentiation of cell substance to maturity is almost if not wholly a matter of selective assimilation once the cell has ceased its divisional activity.

A satisfying example of this phase of differentiation is had in the increase in size and fibrillar definition of non-dividing muscle cells of the regenerating chela of the Hermit crab (68).

It is obvious that the nature of the molecular combinations selectively assimilated for differentiation is determined by the genetic pattern of the reaction basis. Since this is set by the directive factors for differentiation, and since these are as manifold as the patterns they give rise to, it is evident that the molecular combinations which complete their expression are similarly as numerous and diverse. This being so, it follows that while the effective processes for cell maturity are specific and peculiar to differentiation, it is hardly logical to expect to find that any one of them is common to this growth activity wherever expressed.

Objection to this may perhaps be raised on the fact that the number and kind of chemical groups available for protein synthesis is limited and some are common thereto. Now the composition of proteins is infinitely diverse and the differences are constituted by differences in relative percentages of the amino acids which go into their make-up. This diversity comes not only from the permutations afforded by the 20 odd amino acids, but from the fact that these also afford 10 or a dozen different groups and several different types of union (116). That is to say, protein composition is effected not by a single but by a compound reaction. The nature of this compound reaction varies according to the kind and relative percentage of the reactants. This is set in large part by the directive factors for differentiation. It follows then that differences in protein composition are accomplished, not by any single reaction which may be common to protein synthesis, but by the relative participation of the several contributors in the reaction-as-a-whole. Therefore the presence of common groups and the processes they subtend in protein synthesis does not necessarily mean they are common factors for differentiation. All it surely means is that they are common factors for protein synthesis. This is quite another matter as will eventually be demonstrated.

So much for the expressing processes for differentiation.

The enabling processes are something else.

For just as the determinative factors for division differ from those responsible for the mode of partition, so the factors which determine that a new cell shall differ from the old are distinct from those determinative of what the difference shall be. The distinguishment is between

the potentiality for change and the nature of the change: between the capacity for and the quality of differentiation. And each has its own determinants, participants, and reactions. All of which, however, are specific and peculiar to differentiation.

Experimental evidence for the existence of enabling factors for differentiation is had in the observation that the cells of unmetamorphosed amphibians do not go on to metamorphic differentiation unless stimulated thereto by the thyroid (28, 17). That is to say, while these cells can increase in size and number they cannot express their developmental potentialities beyond the larval state until enabled so to do by a specific factor. This is not a species-specific factor because the thyroid of one species will start off the metamorphosis of different quite unrelated species. And it is neither organo- nor histospecific because the organ and tissue products of the differentiation are those normal to the organism in which the reaction is produced; not those of the species providing the thyroid. It is therefore not a directive factor for differentiation, but an enabling.

Now the potentiality for differentiation is at one time or another a common property of all living substance. This naturally leads to the idea that there is a common reaction-basis for differentiation, and the postulation that there exist certain definite and specific chemical groups and arrangements essential for and common to the expression of the activity as a fundamental property. Such a reaction-basis is clearly different from those determinative of cell type and composition specificity—from those which are set by the phylo-, para-, and onto-genetic factors. Because of this; because the expressing factors are interminably diverse; and because the effectuality of this reaction-basis depends on enabling factors, it is reasonable to conclude that there are factors specific and peculiar to differentiation which are also common thereto, and that these are enabling rather than expressing factors.

Experimental support for this reasoned concept is had in a demonstration that one and the same chemical compound acts to bring differentiation potentiality to expression in a variety of organisms which differ widely from each other with respect to species, cell types, tissues, organs, developmental products, and composition. Thus l-proline, a naturally occurring amino acid of general distribution has been found to enhance differentiation expression in Obelia (67), Drosophila (113), Pagurus (70), rabbit ova (88), and spontaneous tumors

of the mouse (61). Other sustaining evidence is available but its presentation will be reserved for the section on amino acids.

So far attention has been focused on cellular differentiation or the factors and processes concerned in those changes in cell substance which lead to its specialization and maturity.

It is now necessary to consider structural differentiation. It is customary to speak of this as 'differentiation'. But the use of this term for this growth expression is a misnomer and conducive to confusion. For structural differentiation is actually a phase of differential growth and development to which proliferation and organization are significant contributors. A tracing of the relation and distinction between this and cellular differentiation will illuminate the point.

Structural differentiation is the progressive development in shape of an organism or part of an organism. It is well shown in the growing point of the shoot and the inflorescence in grasses (21).

It is evident that in multicellular organisms other growth factors than cellular differentiation participate in the picture. Proliferation is a factor on at least two counts. Thus differences in rate of proliferation of the same kind of cells in different localities are important; and in structures composed of different kinds of cells—and there are few which are not—differences in rate of cell increase in number of the several types are undoubtedly of formative significance. Good examples of this are found in the differences in proliferation activity in different regions of kohlrabi and cabbage stems, and the relation of these differences to the size and shape of the stems (77); and in the part played by differential proliferation in the structural differentiation of the regenerating Hermit crab chela (68).

Just as important is the contribution of cell segregation or Organization. As cell lineage is being established in early development, be it embryonic, regenerative, or what, the cells not only differentiate in successive divisions along the line set by the genetic factors, they also progressively segregate and foreshadow in this segregation the organ which they are eventually to form. Like cells segregate with like. And while it is true that the chemical specialization of the cells into like composition, structure, and function lays the foundation for their capacity to organize into the precursor of a specifically functioning structure; cell segregation is a growth activity which is distinct from cellular differentiation, as will presently be demonstrated, and structural differentiation is effected through this segregation as well as through the progressive proliferation and differentiation of

the component cells (68). In other words structural differentiation is not so much a matter of chemical specialization of the cells as it is of the relative increment and arrangement of the chemically specializing cells into a definite pattern. In other words it is not differentiation per se, but it is a growth expression to which differentiation is a considerable contributor. It may of course be used as an index of differentiation providing the choice of indicators is more suitable to this growth activity than to organization or differential growth. Examples of such use may be found in the proline studies with Obelia (67) and Pagurus (70).

It is useful to distinguish between structural differentiation, differential development, and differential growth. It will be instructive to do this on the basis of time, space, and mass.

Now time may be stated to have two qualities. The qualities of time are sequence and duration.

And space can be stated to have three qualities. The qualities of space are dimension, surface which has the attributes of inclination, form, and contour; and the third quality of space is volume.

And mass can be stated to have but one quality. The quality of mass is weight. An attribute of mass is density which is compounded of weight and volume.

Structural differentiation is expressed by sequence of changes in space. It is exemplified in the morphogenesis of the growing part of the shoot and the inflorescence of grasses (21), and the sequence of changing surfaces of humans from embryo to adult (16).

Differential development is expressed by relative rates of change in dimension and volume of parts in terms of the whole or any appropriate standard, and in it time as duration is primary. It is exemplified in the relative increase in length per unit time of humerus and femur, and tail and spinal column in rats (31, 30); the relative increase in length per unit time of the skull, body, and tail of Gar (69); the relative increase in volume per unit time of the several parts of the brain (18); and the relative change in dimensions of the several segments of the regenerating Hermit crab chela (80, 73).

Differential growth is all that is comprised in differential development plus changes in mass on time. It is therefore expressed by relative rates of change in weight, dimension, and volume of parts in terms of the whole or any appropriate standard. It is exemplified in the relative weight increments per unit time of the various organs of the rat under normal (19) and abnormal (32, 81) conditions.

This exposition should make clear the desirability of precision of statement when discussing the growth activity of differentiation. It is also clear that the isolation and identification of differentiation as cellular specialization in such growth expressions is precarious, and that test material for this purpose must be carefully selected.

For this reason and for the reasons given in the discussion of Initiation, it happens that none of the cited organisms save Obelia is at one and the same time adequate for quantitative estimation of differentiation, and also conveniently suitable for exploration of the part a given amino acid may play in this or any of the other basic growth activities.

6. *Organization*

The next step in developmental growth progression is Organization. Every biologist has a mind's eye picture of this growth activity. But ask him to bring it into focus for definition and interpretation and the result is indecision. This comes in part from the fact that organization deals largely with the growth changes of groups of cells instead of those associated with single cells where exposition is relatively simple as in the case of Initiation, Proliferation, and Differentiation; and in part from the fact that frequently the distinction between organization and differentiation is unrecognized in interpretation of results, and these are attributed to the former when actually they are primarily subtended by the latter. Because of this an attempt will be made to establish a concise but comprehensive statement of what organization is on the basis of present knowledge.

A first approximation might be: Organization is cell segregation. This, like most over-simplifications, is incomplete. It fails to take into account the fact that segregation of unicellular organisms such as bacteria, algae, and protozoa, may not be expression of formative growth even though such aggregates may take forms which are roughly species characteristic. Contiguity is of course a natural result of one cell becoming two, two becoming four, four becoming eight, and so on; and the taking on of characteristic form by such aggregates may be as much due to species-specific chemical composition; the physical states produced thereby; and the inter-reaction of these with the medium in which proliferation is taking place, as to any definitive factor for formative growth. In other words the forms taken by such aggregates is quite as probably a result of the composition and physical state of the components as it is of organization

as a specific expression of growth activity. The form may be the result, not the expression of growth. On the other hand neglect must not be made of the probability that organization as a function of growth does play a part in producing the strikingly beautiful forms taken by such colonial-living protozoa as the Volvocidae.

The same indecision applies to interpretation of the forms which the cell substance of cells of multicellular organisms takes as it becomes specialized through differentiation. There can be no reasonable doubt that differentiation sets the composition and that once this is set the subsequent addition and consequent segregation of cell substance according to the composition is the result of selective assimilation. But whether or not the forms taken by the segregating substance are expressions of organization as a directive growth activity, or are simply or largely due to the chemical pattern of the differentiation determined composition is a question. To be specific: We attribute and justly the cubical form taken by crystalline sodium chloride aggregates not to any extrinsic organization factor but to properties inherent in and peculiar to the constituent molecules. Therefore it seems just to conclude that intrinsic molecular properties of myosin and its allied compounds may similarly be as largely determinative of the forms found in muscle cells as are any as yet undemonstrated growth factors of organization. And so on similarly. Where to draw the line and say, that here organization is effective and there, chemical composition and physical state are responsible, is in many cases impossible. Such being so the simple statement that organization is cell segregation is obviously inadequate.

A second approximation might be: Organization is ordered cell segregation. This is better than the first in that it carries the implication of a directive factor and thus eliminates confusion with respect to segregations due to natural contiguity of proliferating cells. It does not, however, eliminate the probability that the orderedness may in many cases be determined by chemical composition and physical state rather than by organization as a function of growth. This boils down to the idea that the forms taken by cell substance segregations in multicellular organisms are the products of differentiation and not of organization.

A third approximation might be: Organization is the growth activity whereby cells are segregated in ordered relation into tissues; and as tissues into organs; and as organs into structures; and as structures into organisms.

On the face of it this seems pretty good. To take mammalian development as an example it shows that organization as a function of growth is a participant in the developmental gamut from the segregation of egg cells to form the morula, and the setting apart from this of ecto-, meso-, and entoderm; through the segregation from these of the definitive aggregates of which organs are composed such as the brain and liver; and the combinations of organs such as bones, muscles, eyes, ears, skin and brain into structures such as the head; through these to the unification of cells, tissues, organs, and structures into the complex organism which is man. Thus it adds to the idea of ordered cell segregation the idea that the segregates are brought into ordered relation with each other. And it adds the idea that organization is concerned with formative or structural development.

Despite these expansions the statement is still deficient in several respects. It is not applicable to the segregation of cell substance of cells of multicellular organisms into ordered relation; a matter which is quite distinct from the forms these segregations may take. And it is not applicable to unicellular organisms where segregation is not of cells together into ordered relation, but of the substance of the single cell into a complete and self-sufficient entity. Nor could the organization (sic) of such colonial-living protozoa as the Volvocidae be explained thereby.

Taking these things into account the fourth and last approximation becomes: Organization is the growth activity whereby the ordered segregation of cells into genetically detemined structural patterns peculiar to a given organism is accomplished.

Now Initiation establishes the beginnings of growth; proliferation establishes cell increase in number; and differentiation specificity in cell composition. But these alone are insufficient to produce that coördinated functionable structure which is organism. Without the exercise of the growth activity of Organization they produce but the inchoate cell masses of tissue cultures; the uncoördinated mixtures of highly differentiated tissues of the teratomas; or the aberrant overgrowths of the neoplasms; and so on similarly.

The function of organization then may be stated to be the establishment of organism as organism.

This idea is agreeably illuminated in the Protozoa. Here the ordered arrangement of the diverse specializations of differentiation establishes the single cell as a self-sufficient organism. A striking example is had in Diplodinium ecaudatum. Here organization of cell

substance, according to the genetically determined pattern, has produced in one and the same cell in functional and structural coödination a neuro-motor apparatus; a ventral skeletal area; an oral opening; a caecum, rectum, and anus; and other structural segregations of specific function.

Now the cells of the Protozoa are able to carry on in the single cell all the basic functions characteristic of a multicellular organism. But the cells of multi-cellular forms are limited to special functions (78). In other words the expression of the organismic activities of a multi-cellular organism is not a matter of the single cell, but of combinations of coördinated cell aggregates. This being so the establishment of organism as organism in such forms is not a matter of organization of cell substance of single cells into self-contained units, but of the organization of many cells together into correlated aggregates.

We are now confronted with the question as to how far organization as a function of growth may be considered a factor in the structural arrangement of cell substance of cells of multicellular organisms.

It will be convenient to distinguish between the part organization may play in producing the external form of the cell; in producing the forms taken by the structural components of the cell; and in producing the spatial relations within the cell of these segregations to each other.

It is well known that the shape of a cell is strongly factored by the physical nature of its environment. It can as it grows be molded into a structure of a definite number of faces by the pressure of surrounding cells (83), and it can become cylindrical or flat depending upon whether it grows into a aperture or on a surface (86). While the form, size, and relation of the structural components of cell substance may set the limits to which external shape comes to conform with the physical environment, it is nonetheless true that this is no fixed attribute. This being so no unadulterated claim can be made that the shape of cells in tissues and organs is evidence of organization of cell substance.

It has been pointed out that differentiation is chemical specialization and that chemical constitution can be considered a definitive factor in producing the forms taken by cell substance aggregates. Therefore the fact that such segregations tend to regularity of form is evidence of a genetically determined consistency in differentiation expression rather than of organization as a function of growth.

Even the form taken by the nucleus cannot be attributed in any particular degree to organization. For here not only is the specific chemical constitution a factor, but also the physical forces which are brought into play at the surface of contact of nucleus with cytoplasm must be considered pertinent factors in determining nuclear shape.

Finally, as is known to everyone who has watched cytoplasmic activity in living cells under the microscope or in cinematic record, form is not a fixed but a changing state. While it is true these changes occur within boundaries, the fact of change of form is sufficient by itself to make questionable any idea insofar as these cells are concerned at least, that organization as a growth activity is much a factor in producing the forms taken by cell substance.

While the segregations of cell substance in ordered relation in such unicellular organisms as Diplodinium ecaudatum can be assumed to be an expression of organization, other factors than this can be invoked as contributing to the structural pattern of cells of multi-cellular forms. It is certainly true that the position of the nucleus in the cell may here be factored by the pressure of cytoplasmic activity; and it can be inferred from correlative data that inter-facial forces existing between nucleus and cytoplasm are no inert factors in this respect. Indeed from the pictures made by artificial cells composed of non-living substances (15) one can not help but accept that physico-chemical factors are the chief factors concerned in the structural relations of cell substance. Frankly I am unable to find unequivocal evidence that organization as an activity of growth is here a participant of moment unless one wishes to make the claim that the physico-chemical set-up is itself the product of organization factors. This I am not at present willing to accept since to me this seems to be rather distinctively a product of differentiation. It is all very confusing and uncertain.

Perhaps we can get somewhere if we interpolate the matter in terms of the four chief aspects of living things, viz., direction, substance, state of substance, and structure (57, 62).

If we invoke the principle of transmission and continuity of qualities as set forth in ancient Indian cosmogony (58) it is evident that each aspect develops from the preceding one, and each succeeding member of the series carries along with itself the qualities of the preceding ones, so that whatever place in the sequence each one of them occupies, even so many qualities it comes to possess (89).

Thus substance—which is chemical composition—is determined by

direction, which is genetic in origin. And state of substance—which is physical in nature—is determined by substance, or chemical composition—which in turn is determined by genetic direction. And structure—which is form and spatial arrangement of form—derives from state of substance which derives from chemical composition which in turn is a derivative of genetic direction.

In other words substance is not substance alone but substance of specific chemical composition as determined by genetic direction; and state of substance is not state of substance alone but physical state as derived from the specific chemical composition produced by genetic direction; and structure is not structure alone but form and arrangement of form as factored by the physical state derived from the specific chemical composition resulting from genetic direction.

If we transfer this concept of continuity to the development of cell structure in cells of multicellular forms we find that differentiation is the only growth activity which emerges with clean-cut certainty as a contributing factor. As shown in the preceding discussion cell substance specificity is the product of differentiation, and it is obvious that the physical reaction basis or state of substance by which structure is conditioned is not self-evolving, or even the result of an additional growth activity, but simply the product of the genetically determined chemical composition.

Where organization may come in as an activity of growth is impossible to delineate in this continuity, and as in all continuities, where there is no finality, definition is impossible. These things being so any conclusion that organization is expressed in the ordered segregation of cell substance aggregates in cells of multicellular organisms must perforce be based on analogy and conjecture rather than on direct and unequivocal evidence. The mere fact that cell substance is organized cannot be taken as proof that this is a result of a growth activity specific for organization since it may be a result of differentiation. This is not to deny that organization as a distinct growth activity may not participate, it is only to emphasize that in this particular continuity a separation out of events attributable to organization cannot be made.

Now growth is a continuity and "In continuity it is impossible to distinguish phenomena at their merging-points, so we look for them at their extremes" (23). Therefore in an attempt to distinguish organization we must look for it in some other place than the struc-

tural development of cell substance in multicellular organisms where the activity, if present, merges inextricably with differentiation.

One extreme has already been noted, viz., the establishment of organism as organism by segregation of *cell substance* in ordered relation in such unicellular forms as Diplodinium ecaudatum. Examples of the other, viz., the segregation of *cells* of multicellular forms into functionally coördinated structural aggregates, can now be given.

It may be assumed that organization as an activity of growth is expressed in the ordered segregation and structural alignment of cells of growing points of plants as they differentiate into their several elements from the proliferation matrix (21).

It is also probably a contributor to the structural segregation into leafy branches of differentiating cells of the callus of Nicotiana grown in tissue culture (11).

And it is assumedly the growth activity which brings the differentiated cells of the Three-Quarter stage of Obelia development to segregation into tentacles, mouth, coelenteron and other structures characteristic of the completed hydranth. (49).

The fact that cells may differentiate and still not go on to segregate into structurally and functionally coördinated aggregates is evidence that organization is a growth activity which is distinct from any other and indeed from any activity of living whatsoever. Such incompletion may be found in tissue cultures (8), in tumors (61), in teratomas (1); and in rodent skin (41) and the regenerating Hermit crab chela (46) under stimulation of cell increase in number by sulfhydryl.

One can envisage the possibility of tracking down the chemical participants which are specific to Initiation; identifying the determinants; and tracing the reactions of cell substance which bring this growth activity to expression, and thus open up the chemical groups wherefrom proliferation becomes possible. There is a hint that the amino acid arginine provides a chemical determinant of this process (66); and it is about as well established as any scientific concept can be, that the SH group is liberated into availability for Proliferation stimulation by some antecedent process of Initiation (40).

One can envisage the possibility of tracking down the chemical participants specific to cell division; identifying the determinants; and tracing the chemical reactions in cell substance which accomplish this growth activity, and which thus open up chemial groups wherefrom chemical specialization becomes possible. It can be assumed from

morphological studies and chemical analyses that nucleo-proteins participate in the activity. It is pretty certain that the SH group is an essential determinant. And if it be granted that each process liberates into availability other chemical groups which are specific determinants of succeeding processes, it is probable that the chemical processes of cell division set free pyrrolidone or some group allied thereto or derived therefrom for the enabling of Differentiation (53).

One can envisage the possibility of tracking down the chemical participants specific to cell specialization identifying the determinants; and tracing the chemical reactions which produce the cell substance composition specific to and characterizing of the differentiating cell. That is to say it should be possible to exactly determine the difference in amino acid make-up of different kinds of cells, such as liver and muscle cells, and it should be possible to trace the chemical reactions by which these differences come to expression. The evidence is not inconsequential that pyrrolidone is in some way associated with Differentiation (51).

Now while the pattern of Organization is established long before cells cease dividing or complete their chemical specialization, the laying out of the structural pattern is sequential to proliferation and cell specialization beginning. That is to say, cells must divide and specialize before they can come to be segregated and arranged into structurally coördinated aggregates. Therefore it is reasonable to assume that just as division makes available certain chemical groups wherefrom differentiation of cell substance becomes possible, so this chemical specialization makes available other chemical groups from which organization becomes possible.

The logical assumption is that there are certain chemical groups and reactions which are specific to organization and distinct from those of any other growth activity, or indeed from those of any other activity of living whatsoever. In other words Organization, like the other basic growth activities, has its own set of participants, determinants, and reactions. Sustaining this is the fact that organization of the growing Obelia hydranth in the completed functionable animal with tentacles, mouth, coelenteron, and other structural segregates is specifically forwarded by some chemical group—possibly allied to the imidine—provided by adenine (49) and guanine (55).

Cell segregation is a first step in structural organization. The arrangement of the cells as they segregate into the coördinated struc-

tural pattern characteristic of the organism and part thereof is the next step.

It can be accepted that there are processes specific and peculiar to segregation per se. These may conveniently be designated as enabling processes of organization.

And it can also be accepted that there are processes specific and peculiar to the arrangement of the segregated cells into characteristic structural patterns. These may be designated as the expressing processes of organization. They are subtended by directive factors.

It is indubitable that the direction of organization is determined by phylo-, onto-, and paragenetic factors and their subsidiary cyto-, embryo-, histo-, and morpho-genetic derivatives. These latter, individually and collectively, successively and coterminously determine the direction of cell arrangement into the structurally coördinated aggregates which characterize the individual organism. They are the expressing factors for organization. They are as numerous as the diversity of structural combinations they evoke.

Thus the direction of Diplodinium organization is expression of a cytogenetic factor; the form taken by the morula of the developing egg is expression of an embryogenetic factor; the sheet-like aggregations of connective tissue cells are expressions of histo-genetic factors; and the combining of liver cells, blood-vessels, bile-ducts and other structural components into that ordered relation which is the liver is expression of an organo-genetic factor.

Morphogenetic factors are expressed by the segregation of muscle cells into such diverse structures as the lengthy Pronator radiiteres, the flat Platysma myoides, and the bulky Gluteus maximus. The aggregation of like cells into like organs such as the thyroid in diverse species such as giraffe and zebra is expression of a paragenetic factor, and the combination of organs and parts into a structurally coördinated organism during developmental growth is expression of the onto-genetic factor. Finally the segregation of parts into species-specific correlated aggregates such as the pine and pansy may be taken as expression of the phylo-genetic factor.

The repetitiousness of this presentation is undoubtedly wearisome. But I know of no better way to set forth the point of view that the end-result of the growth activity of Organization is not the expression of a single but of a composite of intercalated factors, each of which has its own part to play in the devolpmental picture and all of which contribute to the establishment of organism as organism.

Turning to the enabling processes for organization, or those presumably concerned in cell segregation as such, two possibilities occur.

Either the groups specific and peculiar to segregation as an activity of growth derive directly from the enabling processes of differentiation, or they derive separate and independent from these and directly from some process specific to division. The first point of view suspects that organization is an immediate product of differentiation beginning. The second suspects it is an immediate product of proliferation.

To be specific: Granting that pyrrolidone is a chemical determinant of differentiation enabling (56), and assuming for the present that imidine (55) is a chemical determinant for the enabling of organization, the question is—is imidine a product of pyrrolidone and hence of differentiation, or is it produced independently and directly from some chemical reaction concerned in cell division, possibly through mediation of SH? It is hardly necessary to point out that imidine may be thought of as arising directly from pyrrolidone, or indirectly from another source as a result of a reaction induced thereby.

The idea that the enabling of organization stems from differentiation rather than from proliferation seems for the present to be the better approximation. The basis is two-fold. In the first place there is evidence that the germ-layers are not absolutely specific as sources of definitive cells (92), for it has been found among other things that typically mesodermal structures may in amphibia be formed by cells which are ectodermal in origin (100). In other words cells organize according to their specialization, not according to their embryonic origin. In the second place there is as yet no evidence that a mass of essentially undifferentiated (indifferent) proliferating cells will go on to organize without first undergoing specialization.

Further speculation will be deferred to a later section. It can here be pointed out however that light on the problem might be gotten by determining the sequence of liberation of specific chemical groups during cell division and early differentiation by *in vivo* analysis under the microscope and spectrometric examination.

Regardless of what the final answer may be it can be assumed that there are chemical groups specific and peculiar to the enabling of organization which also are common to this growth activity wherever expressed, just as such have been assumed to exist for the antecedent activities of Initiation, Proliferation, and Differentiation.

It is not necessary to repeat *in extenso* the argument in behalf of this concept. To refresh the memory of the reader however the two

facts and the correlating suppposition on which it is based will be restated. It is a fact that structural organization is a common growth activity of living things. It is also a fact that there are amino acids and nucleic components which are common to all cells. It would be strange indeed if this common growth activity were not subtended by chemical groups common to cell substance; groups which are specific to the activity as the activity is a specific aspect of growth.

To recapitulate: Organization may be stated to be a distinct growth activity with its own set of determinants, participants, and processes. Like Differentiation it may be said to have enabling and expressing factors. The former are presumably common to all living things at one time or another, probably at the time of differentiation enabling, possibly at the time of early cell division. The latter are cyto-, embryo-, histo, organo-, and morpho-specific, and phylo-, onto, and para-genetic in origin. It may be assumed that the coming-to-expression of organization is contingent upon an antecedent cellular specialization or differentiation.

One gets the impression that the expression of organization is rather more susceptible to disruption than the other basic growth activities which contribute to the ordered progression of developmental growth.

While it is true that emphasis tends to be focused on structural abnormality because it is more noticeable and dramatic than normality-like crime is more newsworthy than good behavior—yet it may also be true that these structural deviations are more often due to failure of organization directly, than to failure of the underlying processes of proliferation or differentiation.

Contributory to this acceptance is the fact that cells may proliferate and differentiate under a variety of circumstances and still not go on to produce the structures which might be expected of them.

Thus cells from the chick embryo heart in tissue culture may go on proliferating and differentiating, even up to the point where they indulge in rhythmic contraction, and still fail to produce a heart despite interminable transferences to successive cultures (8). And cells of rodent skin may produce inchoate cell masses when stimulated to unwanted proliferation by SH (41). And cells of regenerating Hermit crab chelae may, as they proliferate, differentiate into muscle, epithelial, and other cells, but yield a variety of disorganized aberrant overgrowths instead of the to-be-expected structurally coördinated claw (46). And cells of spontaneous mammary tumors of the mouse may proliferate and differentiate into acini even into acini with ductuli

but fail to organize into the ordered structural relation characteristic of the organ (61).

Since in such and similar cases organization fails while proliferation and differentiation seem to be expressed in that adequacy which under other circumstances leads to organization, it is reasonable to conclude that the expression of organization may be more easily thrown out of gear than that of proliferation or differentiation.

What makes the developmental growth clock stop ticking at just this point? Are the factors for organization expression lacking, or are they prevented from exercising their function by extraneous circumstances?

Now cells may be disclocated from their usual course of structural development by pressure (26) as shown grossly by the deformation produced in the feet of Chinese ladies by binding. And the shapes cells produce are factored by the shape of the environment in which they proliferate (86). It is possible then that the flatness of the surface in tissue cultures and the absence of pressure gradients from contiguously developing structures are purely physical factors contributing, but not necessarily decisive, to the prevention of organ formation by proliferating differentiating heart muscle cells from the chick embryo.

These factors can not be invoked as of any particular significance in the case of the disorganized aberrant overgrowths exhibited in some regenerating Hermit crab chelae, however, for these occur in association with and contiguous to parts of the claw which are developing along the usual pattern of structural organization. Here something else must be at work. It is probable that localized trauma is at the bottom of the failure. It is conceivable that this might so dislocate developing groups of cells from their normal relations that their subsequent organization along the line of the to-be-expected pattern is mechanically prevented. Thus trauma would be a preventative of organization expression.

Now it may be assumed that the processes of cytogenesis produce the factors for tissue organization; and that the processes of histogenesis produce those for organ formation.

In other words, it is the inter-reaction of the organization processes of one cell with those of another of the same kind which presumably gives rise to the factors whereby the organization of like cells into a tissue is enabled; and it is the inter-reaction of the organization processes of one kind of tissue with those of another which presumably

gives rise to the factors whereby the organization of diverse tissues into an organ is enabled; and so on similarly for structure and organism organization.

If this be granted it can happen that trauma also acts to produce a lack of factors for organization; for it is conceivable that the breaking of structural continuity through dislocation of tissues so prevents the inter-reaction of histogenetic organization processes that the morpho-and organo-genetic factors are not formed and structural organization according to pattern is impossible. Thus failure of organization after trauma can be taken to arise both from mechanical prevention and lack of essential factors.

Now the usual culture of animal cells contains but one kind of cell: at best it does not contain all the kinds, actually or potentially, which go into the make-up of the organ of which the dominant cell type is representative. It is therefore evident that the complexus of histogenetic processes essential to the production of organogenetic factors is absent and hence that the failure of such cell aggregates to organize as they proliferate and differentiate is contributed to as much by the lack of morphogenetic factors for organization as by the physical nature of the milieu. This deduction is supported by the fact that in plant tissue cultures where different kinds of cells are present in adequate relation it may happen that some of these go on to organize into a leaf form (11). Who knows but what a like organization might be accomplished in tissue cultures of animal cells if proper procedure be devised? The value of bringing to observation such a miniaturization of developmental progression expression for study of the basic processes of growth needs no exposition.

Now there are examples of failure to organize which do not seem to fall in the above noted categories.

Thus the cells of mammary tumors form breast tissue but no breast. They proliferate and differentiate into stromal and acinar masses; but these have no predictable relation and no coördinated pattern of development into a functionable organ (61). It would take quite a stretch of the imagination to attribute this to mechanical distortion of the milieu. The same is true of the polypoid growths induced in the skin by tar derivatives (98) and by simple stimulation of cell increase in number with SH (41).

It is noteworthy that in these and kindred cases the failure of organization along the to-be-expected line is associated with an unwonted proliferation activity. That is to say, a proliferation activity

which is unusual for the time and place: time here being used in the sense of sequence (69) or stage of development of the part in which the exacerbation is induced. Any one or more of three possible factors may be conceived of as participating in these disorganizations. (*a*) It may be that the factors for organization are destroyed. This might hold in the case of the carcinogens. It could hardly hold in the case of SH stimulation because here polyp production is the exception not the rule, and the usual result is just a thicker and more highly differentiated skin. (*b*) It may be that the factors for organization are not produced. It is conceivable that unwonted proliferation could bring about a state in localized areas where new cells are produced faster than they can differentiate. That is to say, the balance between proliferation and differentiation is kept so far to the left that production of organization factors by progression to the right is impeded. This could hold in the case of the tar and SH polyps. It might be less important in the case of mammary tumors where varying degrees of cellular specialization are exhibited. Indeed it may be that the disorganization of mammary tumors is in part due to non-production of organization factors, not because of unwonted proliferation; but because the change in chemical processes which is induced by the acquisition of malignant properties by the cell fails to afford reactions wherefrom such factors may be produced. Supporting this supposition is the fact that differentiation of malignant cells seems to differ qualitatively as well as quantitatively from that of non-malignant, and this can be taken as evidence of a deep-seated distortion of the cellular processes of development. (*c*) It may be that differential proliferation rates are contributors. It is trite to note that different kinds of cells increase in number at different rates. And it is reasonable to assume that these differences are inherent in the cells and not imposed on them from the outside to any great degree during normal development. This assumption has experimental support from the observation that when proliferation is stimulated by SH in the regenerating tail end of Clymenella torquata the rate of increase in cell number is distinctive and characteristic for each of three cell types (43). Furthermore, it is conceivable that the state of differentiation of the cells at the time of stimulation would condition the rate of cell increase in number which the cells would exhibit. In other words, the diverse proliferation potentialities arising from differences in cell types and differences in state of differentiation normally present in a cell milieu, such as the skin and mammary gland,

could—on the introduction of a proliferation stimulus—conceivably give rise to a distortion from the usual of the relative mass of cells which any given type of cell contributes to the increasing whole, and thus become a significant factor in structural disorganization.

To recapitulate: Organization according to plan may fail of expression for a variety of causes. Inadequate surface conditions; pressure; traumatic dislocation; all may act to prevent cell segregation into functionable aggregates. Defects in histogenetic relations may result in lack of chemical groups and factors essential for organogenesis. And changes in cell proliferation activity from the usual which upset the balance between cell increase in number and cell specialization, plus those changes which produce distortion of the relative masses of different kinds of cells in a given growing part may also be effective. The picture as a whole suggests that organization—of all the basic developmental growth activities—is the most susceptible to frustration. Not that it is too easily diverted from its usual course; but that its divagations occur under conditions which do not seem to seriously disturb the other growth activities. It may be that this is more apparent than real. For organization failures are more obvious and dramatic than failures of initiation, proliferation, and differentiation. Indeed these may occur just as frequently but be hidden and unobserved. On the other hand the variability in organ and structure form and the variability in distribution of the constituent tissue elements therein, are indications that while the general genetic pattern produced by organization is maintained within limits, these limits are decidedly flexible. The wonder is, not that organization expression is variable; but that, in view of its relative sensitivity to disturbance, it is not more often distorted from functional usefulness.

Since failure of organization is a characteristic of malignancy it follows that understanding of organization is a prerequisite to understanding of cancer as a growth.

If one wished to split hairs one might claim that the growth of a cancer is organized according to the laws of cancer growth, and that cancer growth only "seems" to be unorganized because the spatial arrangements of the cells comprising the cancer are not those usually taken by these cells in the production of a non-cancerous structure.

The point of view depends on the frame of reference. If one takes the stand that the transformation from body cell to cancer cell in-

duces a change in the genetic constitution of the cell, and such could justly be held since cancer cells breed cancer cells, then it could be held that the genetic determinants of cancer growth differ from those of non-cancer growth, and hence that the organization of cancer cells might naturally be different from that of non-cancerous cells. If on the other hand one takes the stand that the transformation of body cell to cancer cell produces a change in timing of the chemical processes concerned in developmental growth, with the result that the different proliferation rates of the different kinds of cells, and the subsequent progress of differentiation is both relatively and absolutely distorted; then it is possible to accept that the failure of cancer cells to organize along the lines of non-cancerous cells may be directly factored by dislocation or lack of the chemical factors specific and essential to the expression of this growth activity.

This latter point of view has support in the fact that in mammary cancer where isolated groups of acinar cells differentiate up to the stage which is comparable with that obtaining in normal breast tissue, there these cells may organize into acini which are quite like those found in the normal breast. Cells which do not so differentiate do not so organize.

There is a real distinction between the two points of view. The first supposition holds there is organization, but organization which differs from the to-be-expected because a shift in genetic composition establishes a new pattern. The second supposition holds there is no organization, no pattern, and that this is because the ordered sequence of developmental progression is disrupted when body cell becomes cancer cell.

This is a problem for the biologist to solve. And it is important enough to deserve his concentrated attention. Solution will be no simple task. It will require integrated inquiry using all present and future resources of genetics, chemistry, physics, and anatomy (62, 60). When we find out why cancer cells fail to organize as non-cancer cells do we will also find out a lot about organization. The abnormal is a sign-post pointing to understanding of the 'normal', just as the 'normal' is a sign-post directing us to understanding of the abnormal.

To summarize then, it can be stated that organization is the growth activity whereby cells become segregated according to a definite but somewhat flexible pattern; that the pattern is determined by genetic factors which are as yet unisolated and unidentified (115); and that the expression of organization may be conceived of as accomplished

by specific chemical groups, factors, and reactions which are produced by and derived from the antecedent growth processes of differentiation.

A specific example may illustrate the latter, viz., it is conceivable that as tendon and muscle cells arise from mesenchyme cells by differentiation the one develops a component *'T'* which characterizes the cells as tendon cells; the other develops a component *'M'* which characterizes the cells as muscle cells; and both develop a common component *'C-C'* whereby jointure between tendon cell and muscle cell to form muscle is effected. And so on similarly for the jointure of different kinds of cells into organs and for structures.

The *'C-C'* component does not determine the direction of organization any more than the coupler component of freight-cars determines the structural make-up of the freight-train. All it is is a specific chemical mechanism for jointure or accomplishment of organization. The arrangement of the cells to form an organ, like the arrangement of cars to form a train, is determined by quite other factors. In both the number of each of the different kinds of units used for organization is similarly governed. In other words the factors which direct the structural organization of cells into an organ or structure are to be distinguished from those which establish the jointure. The latter are specific and peculiar to organization and presumably common thereto wherever it is expressed. The former are genetic in origin and nature and differ according to the genetic constitution of the organism in which they are exercised. They are none the less specific and peculiar to this growth activity.

This long dissertation has shown if it shows nothing else the unsurety which must accompany any attempt to isolate and measure the locus and degree of participation of the growth activity of organization in most developing organisms. Derived as it is from the processes of differentiation and compounded as it is of a regulated cell increase in number there cannot help but be uncertainty as to whether or not it is organization which is affected by a given experimental procedure. Hesitation can only be resolved when there is first used an organism in which the expression of organization is measureably separable from that of the other basic growth activities. Of all the test materials cited in preceding paragraphs Obelia is the only one which fulfills this requirement within reasonable limits and is at the same time conveniently suitable for experimental explorations. This will become apparent as the presentation develops.

7. *Substance Increment or Anabolic Growth*

So far I have dealt with the developmental aspects of growth: or the growth activities wherefrom the identity of organism is accomplished. Now I shall consider the mass increment aspect of growth; or the growth activity whereby living things increase in substance and size.

There should be no need to give examples of growth by increase in mass. They are everywhere apparent. They are the common subject of comment, study, and anxiety of parents, gardeners, and breeders. There is hardly a plant or animal whose increase in weight or dimension on time has not been charted and mathematically analyzed. Great compilations have been made (6). In fact the accumulations of data have been so voluminous that they seem to have smothered any consideration of the idea in the minds of the recorders that the growth of an organism from its beginning to its functionable maturity is not a matter of substance increase alone, but of this plus cell increase in number, cell specialization in composition, and cell segregation into structurally coördinated aggregates. Growth is not single but multiple—the summated expression of integrated developmental and incremental factors and processes (47). Perhaps this blindness to the obvious is due to the fact it is much easier to put weights on a balance or stretch a tape measure, and much more convincing of erudition to show familiarity with exponential equations, than it is to follow through the changes which cells undergo as they contribute to the production of an organism, and to delineate this in terms of absolute and relative increase in cell number, differentiation, and organization.

Now everybody will admit that an organism may increase in weight and/or size long after its pattern has become established by the combined expression of proliferation, differentiation, and organization. And conversely it is a matter of record that groups of cells may under certain conditions divide, specialize, and express some degree of ordered segregation, without apparent increase in total mass. It would seem reasonable to assume therefore that there are chemical processes productive of mass increment which are distinct from those productive of proliferation—or distribution and separation of cell substance; distinct from those productive of differentiation—or compositional specialization of the dividing cells; and distinct from those productive of organization—or the segregation and ordered arrangement of the chemically specialized material. That

is to say, the increase in number of living units; the increase in kind of living units; and the increase in arrangement which these living units may take, are one and all growth expressions which are distinct from the increase in the amount of dividing, differentiating, and organizing substance. All are necessary to the production (growth) of a completely functionable organism, and no distinction can be made as to their relative importance. No one can be singled out as the specific index of growth. And all must be taken into account when evaluating the growth reaction of an organism to any experimental procedure.

It is well to distinguish between substance increase which is constructive and that which is storage. The processes which produce the former are growth processes. Those which produce the latter are not. While it may not be now possible to separate the two in fact, it is essential that the two be separated in concept.

Constructive increase is shown in the mass and size increase of newly divided cells; the increase in fibril size and substance as immature muscle cells go on to maturity; and in the segregation and ordered deposition of cellulose, chitin, and calcium salts as skeletal forms. It therefore may be taken to be the incorporation of additional substance as part of living cytoplasm and those products thereof which contribute to the functional and structural specificity of the organism. It is in essence that increase in substance wherefrom derives expansion of functional and structural capacity according to kind. It is thus seen to be the usual accompaniment of the basic developmental growth activities and is undoubtedly a significant contributor to the accomplishment of their expression.

Storage increase in substance—which is not growth—is exemplified in the accumulation of starch granules in plant cells and glycogen in animal; in the concentration of specific protein in seeds of corn and wheat; and in the deposition of fat resulting from over-eating, endocrine imbalance, and the like. It therefore may be taken in general to be the extra-cytoplasmic deposition of structurally unessential non-living matter from plethora. It is in essence increase in reserve supply of energy providing material for cytoplasmic activity as distinct from increase in active cytoplasmic substance itself. The processes concerned in such substance increase are obviously different in part from those concerned in constructive substance increase.

These things being so the use of weight increase alone as an index of 'growth' may be treacherous procedure indeed. Personal experience

has been that the heavier of two rats of equal age and body length is frequently heavier because there were large accumulations of fat in the visceral and other fat depots and not because the individual organs were correspondingly heavier. The conclusion is that just because one animal has gained more weight per unit time interval this is no proof it has grown faster. It may be that it has stored more fat. Such fat storage was a considerable contributor to the greater weight of the so-called superior strain of Wistar rats developed by Greenman and Duhring (27).

Weight increase is not always growth and all weight increase is not necessarily constructive. This is all too evident in the abdominal and other enlargements of human middle-age.

It would therefore seem advisable to combine with record of weight increase, a record of that dimension increment which is essentially free from storage factors—such as body, tail, and limb length—and wherever possible with estimation of free fat. Certainly interpretation of experimental weight increase as growth without keeping in mind the distinction between constructive substance increase and storage substance increase is potentially productive of error, since it may not be growth at all which is directly affected, but some phase of intermediate metabolism (52) wherefrom storage ensues.

Now cellulose, chitin, the calcium phospate complex of bone, and other structurally essential substances do not, as far as we know, produce living cytoplasm; but living cytoplasm as far as we know, is capable of producing these materials. It is therefore appropriate to consider constructive substance increase in living cytoplasm as primary, and constructive substance increase in framework compounds secondary.

Granting this it follows there are two types of constructive substance increase, viz., increase in amount of living cytoplasm, and increase in amount of the structurally essential products thereof. Among these should be included the panniculus adiposus and other aggregates of adipose tissue of formal function. Obviously the processes which yield the former are distinct from those which produce the latter. That is to say each has its own determinants, participants, and reactions.

It may then be assumed that there are processes which are specific and peculiar to cellulose formation which are common thereto wherever this is exhibited, and that the same is true for the formation of

chitin, bone, and the other secondary expressions of constructive substance increase.

Extending this principle to increase in mass of living cytoplasm it may be assumed that there are processes specific and peculiar thereto which are distinct from those concerned in any other basic growth activity, or indeed in any other activity of living whatsoever, and that since the essential components of cytoplasm are everywhere the same, there are specific and peculiar processes for increase in cytoplasmic mass which are common thereto wherever this is expressed.

It has been suggested that the growth activity of present discussion be called anabolic growth (47). It could perhaps as well be called assimilative growth were it not for the fact that the word assimilate carries the meaning 'making similar' as well as the meaning 'inbuilding'; while the intension of the word anabolism is customarily single and restricted to 'inbuilding'.

It would therefore seem preferable to use 'anabolic growth' when concern is solely with the processes by which inbuilding is accomplished, and 'assimilative growth' when concern is not only with these but also with the factors concerned in the selective assimilation of substance specific and peculiar to the kind of cell undergoing mass increase.

We thus come to distinguish between the act of increment and the kind of increment; between the factors which determine that anabolic growth may and shall occur, and the factors which determine what shall be used for its expression.

That anabolic growth may occur is due to chemical groups and combinations comprised in living substance. That it does occur is due to properties peculiar to such compounds in combination in living substance.

Since the reaction basis of living, of which growth is an expression, is largely protein, exposition will be based on this cytoplasmic element with the implication that the principles are applicable to other components of the living matrix.

Now the basic components of the proteins are the alpha amino acids. These are characterized by the common possession either actually or potentially of carboxyl and amino groups in close juxtaposition. The hydrolytic jointure of these groups into peptide linkage is a fundamental and universal process whereby amino acids are built into protein. While other groups may and probably do form

other types of linkages, present information indicates that the peptide union is basic and common to all protein production (103).

That this protein construction linkage shall occur depends either upon a specific chemical agent and/or physical state which is the peculiar product of compound in combination in living subtance. This too can be taken to be fundamental and universal.

Clearly then the amino and carboxyl groups of cytoplasmic and other protein and their jointure in peptide linkage are basic factors which determine that primary anabolic growth may and shall occur.

The commonality and specificity of these reactants and events is ample basis for the postulate that primary anabolic growth has determinants, participants, and reactions which are not only specific and peculiar thereto, but which are common to its expression wherever this may occur.

Indeed the existence and extent of the direct evidence in support of the applicability of this principle to this growth expression strengthens the belief it is similarly applicable to the other basic growth activities.

Although the basic bond by which amino acids are combined into cytoplasmic protein is everywhere the same, the amino acid composition is not the same for all cells. The differences are due not so much to differences in kind of amino acids as to differences in the relative number of each kind and their arrangement in the molecule. It is therefore well to consider not only the factors which determine how substance increase in cytoplasmic protein is brought about, but also the factors which determine the make-up of this added substance.

It was postulated earlier that the chemical specialization of cells is set by the genetically determined factors of differentiation; that this occurs as cells undergo division; that it is largely accomplished by cytoplasmic neo- and re-constitution; and that subsequent increase in substance according to kind is a product of selective assimilation.

It is a nice question whether selectivity in assimilative growth is determined by directive factors for differentiation, or by expressing factors founded in the particular chemical composition which differentiation has established in the substrate. That is to say, whether differentiation determines only the substrate, and the substrate determines the subsequent increment; or whether the factors for differentiation directly determine both the substrate composition and the relative amounts and arrangements of the amino acids taken from the

milieu and inbuilt into the cytoplasmic protein undergoing anabolic growth.

Inclination is towards the first possibility since cells do specialize without apparent increase in substance. This should not occur if the same factors govern the selective assimilation of anabolic growth and differentiation, since if this were so the former would be the invariable accompaniment of the latter.

These distinctions may seem finely drawn; but just as it is necessary to know the structural components of an organism in order to understand it as a whole, so is it necessary to resolve growth into its components if it is to be understood as a whole.

To summarize: Increase in substance of living things is expression of anabolism. This activity is substended by processes which are specific and peculiar thereto. The increase may be either constructive or storage. Storage increase in substance is not a growth activity, but simply an accumulation of reserve materials from plethora. Constructive increase in substance is a growth activity. It is primary anabolic growth when it consists of increase in amount of living substance. It is secondary anabolic growth when it consists of those products of living cytoplasm which are structurally essential, such as cellulose, chitin, and bone. Distinction is made between factors whereby increment is accomplished, and factors which determine what the composition of the added substance shall be. The latter are as diverse as the differences in chemical composition of the cytoplasmic substrate which increases substance according to kind. While this is true, it is also true that the peptide linkage is probably a universal mode of jointure between amino acids as they combine to form cytoplasmic protein. This justifies the postulate that there are determinants, participants, and processes which are not only specific and peculiar to the accomplishment of primary anabolic growth, but which are also common to this activity wherever it is expressed. The factors for secondary anabolic growth however are the products of cellular chemical specialization and consequently are as diverse as the substances they subserve. Yet it can be postulated that there are factors for substance increase of any given secondary product—such as cellulose—which are not only specific and peculiar to the production of the particular growth component, but which also are common thereto wherever it may be expressed. The same would hold true, of course, for any commonly occurring product of common basic composition.

Anabolic growth is measured in weight and/or dimension change. Note has already been made that the use of weight increase alone is no sure index of constructive growth since storage increment is frequently a contributor to such increase. The use of dimension increase alone is also fraught with danger. This is particularly true when an attempt is being made—as it should always be made—to evaluate the significance to growth of any given compound or procedure, not in terms of growth taken as a single activity, but in terms of the basic activities of which growth is a combined expression.

To take a relatively simple example. Increase of roots in length is factored both by cell increase in mass (size, length) and cell increase in number (38). Each of these growth expressions has its own set of underlying chemical processes. Each has potentiality for separateness of reaction. It is conceivable that length growth of nondividing cells may increase without concomitant increase in cell division; and conversely that proliferation may be stepped up without acceleration of cell increase in size. This latter has experimental demonstration. Thus roots exposed to sulfhydryl grew longer than their controls. Sections showed increased mitotic activity but no increase in cell length. The SH-treated roots were therefore longer, not because anabolic growth was directly stimulated, but because the substance increase was determined by a primary stimulation of cell increase in number or proliferation (40).

It is therefore evident that dimension increase may be factored by other basic growth activities than the anabolic. This should be taken account of in interpretation. Particularly by the auxinites and nutritionists.

Now most any organism which can be conveniently weighed or measured is suitable test material for anabolic growth if interest is only in the amount of substance increase. But if interest is in the factors peculiar to anabolic growth as distinct from the other basic activities, then an organism must be chosen in which this can be separately estimated. This requirement is met by the colonial-living form of the marine hydroid Obelia as this monograph will demonstrate.

It has long been known that nitrogenous compounds are necessary components of living substance. And it has long been held that the wear and tear of living entails a breakdown and loss to the organism of such components. Since there is no appreciable progressive decrease in body protein under usual conditions it has been assumed that this is renewed as fast as it is destroyed and that nitrogen compo-

nents of the ingesta are the substances used in this renewal. It is of present interest to determine if this renewal is a function of anabolic growth. Before this can be decided it is necessary to determine whether the concept of destruction through wear and tear is well taken.

The idea derived from the fact that nitrogen is excreted in the urine even when no protein is present in the diet (22). But the conclusion may have erred on two counts. It tacitly assumed that the nitrogen came from destruction of cytoplasmic protein and neglected the possibility it might have come from non-cytoplasmic protein. And it assumed that a result obtained under the unusual condition of essential nitrogen starvation was representative of a reaction taking place under usual conditions of adequate nitrogen intake.

There can be no question but that nitrogen exchange is a necessary accompaniment of living. But that break-down of living cytoplasmic protein is a step in this exchange may now be questioned.

Recent studies with tagged atoms have shown that dietary amino acids may enter the protein molecule and replace the same type of amino acid in the proteins, viz., leucine may replace leucine, etc.; that there is a constant wandering of alpha-amino nitrogen from one amino acid to another in proteins; and that nitrogen may be transferred in proteins from the amino group to the amidine in such amino acids as arginine. Similar exchanges occur in the purine components of nucleo-proteins. "These processes are so balanced that they do not lead to an ultimate change of the system, i.e., of the total amount or composition of the cell" (99).

From this it would seem as if nitrogen exchange under usual conditions is just that and nothing more, and that the ancient shibboleth that the activities of living determine a destructive wear and tear of basic cytoplasmic protein can be thrown into the limbo of one-time useful but now outmoded hypotheses.

Granting this there is no need to assume that anabolic growth is here concerned.

Quite otherwise can be the case in conditions of nitrogen starvation. But even here it is not necessary to demand that the lost nitrogen represents destruction of cytoplasmic protein. Cells contain other protein than this. Cytoplasmic protein can be taken to be restricted to that protein which constitutes the actual reaction basis of living—the acting, not the acted upon; and it can be assumed here is also present a labile protein of loosely bound amino acids—the product of secondary anabolic growth which is the acted upon

but not the active. Keeping in mind that nitrogen exchange is a necessary event of living, it can be assumed that cytoplasmic protein draws upon the nitrogen of this labile protein rather than that of its own substance when and if nitrogen starvation is induced. This idea is supported by the fact that whereas bodies may shrink and cell contents become tenuous in conditions of complete starvation, always is there left at death a greater residuum of potentially active cytoplasmic protein than would be expected if this was being self consumed. Death from starvation then would come not from utilization of cytoplasmic protein, but from exhaustion of the accessory protein which provides in times of nitrogen starvation the nitrogen groups necessary for the nitrogen exchange activity of living.

Granting this analysis to be essentially correct it would follow that secondary anabolic growth may well be responsible for the rebuilding of labile protein when nitrogenous compounds are readmitted to the diet subsequent to non-lethal starvation. But it may be emphasized that this is an event determined by unusual conditions, and not one common to conditions where nitrogen intake is adequate for the needs of nitrogen exchange.

The concept of destructive wear and tear could also have been objected to at the time of its proposal because of the difficulty of envisaging a simultaneously occurring destruction and reconstruction in the reactive matrix of one and the same cell. While this objection might appear to be resolved by the later observation that cells do have periods of relative relaxation from functional activity; as is intriguingly shown in the going out of action of groups of renal glomeruli as others come into action (97), and the hesitations and apparent recessions in the growth progression of an Obelia hydranth (7), the alternative postulate that there is no wear and tear but only exchange is personally preferable in the light of modern research.

So far dealing has been solely with intra-cytoplasmic activity.

There is however a body protein loss which beyond peradventure is due to the ordinary wear and tear of living. Such is found in blood-cell destruction, epithelial cell desquamation and the like. But such loss is peculiar to the type of organism. And replacement is a matter of new cell production by special organs, tissues, or cells. Since this replacement is subserved by the basic growth activities it is clear that anabolic growth is a necessary participant therein.

To summarize: While the ordinary exercise of intra-cytoplasmic functional activity determines the utilization of nitrogenous com-

pounds, this may be assumed to be a matter of nitrogen exchange between the nitrogen containing groups of the ingesta and those of cytoplasmic protein rather than a destruction and rebuilding of this cytoplasmic constituent. Such being the case no assumption should at present be made that anabolic growth is here concerned. On the other hand this growth activity can be assumed to be responsible for replacement of protein lost when cells are destroyed or cast off in the wear and tear of living, since the renewal of these elements is a property of the basic growth activities. It is also probable that anabolic growth is responsible for replacement of non-cytoplasmic protein utilized in conditions of starvation. And so on similarly for the exchange and replacement of other substance.

8. *Growth vs. Metabolism*

So far the discussion has dealt only with growth as such. But growth is not an isolated activity. Its processes are coördinated and integrated with those of other activities. To trace all these relations is beyond the scope of this introduction. It is germane however to examine the distinction and relation between growth and that which is called metabolism.

Before going on to this however it will be just as well if the meaning of metabolism is clarified. The undiscriminate use of the word has brought it, like the Atlanta *Journal* which covers Dixie like the dew, to cover every imaginable chemical reaction which the living organism is capable of.

According to this intension the chemical reactions of growth have to be considered as expressions of metabolic activity, and growth is to be taken as an aspect of metabolism.

On the other hand it can justly be held that growth is a factor productive of metabolic activity (109), and hence that metabolism in some part is an aspect of growth.

Such merry-go-round thinking gets nowhere. The dilemma can be resolved however by restricting the coverage of the word metabolism to the chemical reactions concerned in substance utilization for maintenance. By so doing the word reverts to its original meaning, and comes to cover a particular expression of living instead of one which belongs to all activities.

It might be a good thing to do this anyhow since the word metabolism is etymologically incapable of standing for the meaning ascribed to it by current usage. The root *meta* means away, after,

beyond, and between; and the root *bol*—means put and throw (87). By no stretch of the imagination can any combination of meanings carried by these roots be brought to stand for the chemical reactions in, by, and of the substance of living organisms.

Since in last analysis the concern of that which is called metabolism is nothing more nor less than the production, utilization, and expenditure of energy for the exercise of all living,—and since this is largely provided by chemical change and exchange of substance in, by, and of living organisms—the roots of any term used to express such a general activity should at least approximate in meaning the basic attributes the word is intended to stand for, viz., substance and energy.

Such may be obtained from the word *plasma* meaning the thing, stuff, or substance moulded; and the word *ergon* meaning work or energy. *Plasma* is appropriate because of its restrictive association with living things; and *ergon* because it may represent all manifestations of energy be they chemical, electrical, thermal, or mechanical.

By combining the two into *plasergy* a word is had whose roots carry the meaning of work done in and by the substance of living. This can be extended without strain to stand for the chemical reactions involved in energy production, utilization, and expenditure in and by living organisms; and conversely for the energy exchange involved in cytoplasmic activity as derived from chemical changes and exchanges in substance. *Anaplasergy* would suitably cover substance increase; *cataplasergy* its breakdown; and so on similarly for other useful terms (63).

Ordinarily one does not look with hospitality upon the coining of new terms; but in this case it does seem as if rectification of an ancient wrong is indicated. The admission of plasergy and its natural derivatives can be objected to on three counts, viz., it is new; it upsets custom; and it does away with a fine honey-dripping word. It can be advocated on three counts. It brings word symbol and subject together. Like other terms of general intent such as growth, reproduction, respiration and the like it implies the product as well as the processes of activity. And its phonetic quality is consonant with its intention. In other words the word means something and sounds like what it means. With it there can properly be had a *Journal of Plasergic* (Substance Utilization) *Research,* instead of improperly having a *Journal of Throw Away* (Metabolic) *Research.*

With this digression out of the way attention can now be given to the distinction and relation between growth and maintenance.

Now maintenance comprises nothing more nor less than the chemical reactions which provide the energy exchange essential to aliveness. It is in essence the utilization of substance for the upkeep of the functional activity necessary to existence. While aliveness is necessary to growth, growth is not necessary to aliveness. It is evident that growth and maintenance may be taken as separable activities. The same types of substance are used for maintenance as for growth, namely fats, proteins, and carbohydrates. In the first case substance is consumed, destroyed, and exchanged to provide energy for the activities essential to living. In the second it is conserved, arranged, and built into the organism to provide the matrix wherewith the activities of living may be expressed.

No growth is glucose and fat combustion; the deamination and nitrogen exchange of amino acids; and that which is called specific dynamic action. Concisely the concern of maintenance is the production of energy; the concern of growth the accumulation and arrangement of substance wherewith energy may be expended. This then is the distinction between growth and that metabolism which is maintenance.

Such being the case it is just to assume there are determinants and reactions specific and peculiar to maintenance which are distinct from those of growth and indeed from those of other activities. And since maintenance is an activity essential to all living it can be postulated that there are determinants and reactions which are not only specific and peculiar thereto but also common to the activity wherever it is expressed.

The relation between growth and maintenance can be briefly demonstrated. The simplest example of relation is the effect of inadequate food intake on the growing organism. Here maintenance continues essentially as usual while growth in substance is either retarded or stopped entirely. Such differential reaction is seen in undernourished plants and animals.

Insofar as growth is concerned this is a matter of obstruction to substance increase by diversion of substance from this activity to that of maintenance, and not a matter of obstruction to substance arrangement such as occurs in differentiation and organization.

This conclusion comes from the fact that whereas plants and animals may be stunted with respect to size growth when food intake is insufficient, they may nonetheless go on to differentiate and organize almost if not completely as their more adequately fed congeners. In

other words they are simply miniature but complete editions of their better nourished fellows. This is further evidence that growth is an aggregate of separable activities each of which has its own set of processes, participants, and determinants; and that the processes of substance increase are not necessarily involved in those of differentiation and organization.

The relation between growth and maintenance, then, boils down to the fact that the substance needs of aliveness take precedence over those of anabolic growth, with the result that when substance is insufficient for both it is diverted from use in anabolic growth to use in maintenance, and constructive substance increase is slowed or stopped while energy expenditure for living continues. That is to say, maintenance takes place at the expense of anabolic growth when and if food intake is inadequate; and conversely, the extent to which anabolic growth is expressed is dependent upon the amount of substance left over from that required for maintenance. This is evidence that self-preservation is a basic law of nature, be it conscious or unconscious.

To summarize: growth is distinct from maintenance (metabolism) in that the former uses substance for mass increase while the latter consumes it for energy; and growth is related to maintenance in that substance utilization for mass increase is directly secondary to substance utilization for the energy needs of aliveness.

Maintenance activity is usually estimated, not by changes in weight, size, or structure, but by chemical determination of the respiratory quotient (118) to which data on nitrogen exchange are added as checks when feasible (3). Such analyses can and have been made on a wide variety of growing organisms (91). In order, however, to be able to correlate maintenance expression with that of each of the basic growth activities to best advantage it is necessary to use test material in which this and these can be simultaneously and separably estimated without interference with the ordered progress of growth. While Obelia, as has been said, is eminently suitable for the latter, its suitability for the former is restricted by the fact that maintenance activity is estimated therein from structural change rather than from chemical analysis (50). The nature of the change, and its validity as an index of change in maintenance activity will be given in the following paper.

D. General Summary

This introduction was designed to demonstrate that if one wishes to determine the influence of any chemical group or compound on growth, it is first necessary to analyze growth into its components, and then to use as test material an organism in which each of the components can be separately estimated.

In it growth is taken to be not single, but multiple—the summated expression of integrated developmental and incremental factors and processes.

These are said to be grouped into five basic activities, viz., Initiation —or the intracellular changes incident to New Growth production; Proliferation—or cell increase in number; Differentiation—or cell specialization in chemical composition; Organization—or cell segregation and arrangement into structural aggregates; and Anabolic Growth —or constructive increase in mass or substance. Intercalated with the latter is the basic non-growth activity of Maintenance—or the utilization of substance wherefrom energy is provided for the upkeep of existence or being.

It is held that each of these basic activities has its own set of determinants, participants, and reactions which are not only specific and peculiar thereto but also common to the activity wherever it is expressed.

While the property and function of each activity are taken to be distinct from those of any other, the processes of each derive from and are coördinated with those of the others.

In other words just as an organism is a whole composed of parts each with its own identity, yet each related to the others, and through this to the whole; so is growth a whole compounded of separate activities, each of which has its own identity, yet each of which is related directly or indirectly to the others, and through this to the whole.

Since each activity is obviously capable of separate reaction, it is held that the significance to growth of any given chemical group or compound is determined not by its influence on growth as a whole, but by which of the several basic activities is specifically affected thereby.

This being the case it is maintained that first evaluation of the part a given chemical group or compound plays in growth, depends upon the use of test material in which the several basic activities are distinguishably expressed.

It is stated that the colonial-living form of the marine hydroid

Obelia meets this requirement adequately. Demonstration is to be found in the data of the following pages.

Even the casual reader will know that each of the chief subjects discussed here deserves expansion into a book. This duty is left to those who come after. Such being the case it follows that the references here given are but a sample of those possible. The choice has rested as much upon personal acquaintance with the worker, admiration for the work, and personal fancy, as upon logical selection of most pertinent data. As may be noted the references tend to be of work which suggests new lines of thinking, of work which is comprehensive in point-of-view and accomplishment, and of work which has contributed to the foundations on which we build. Such must not be lost to sight in the dust of contemporary publications.

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CONSERVATION OF SCHOLARLY JOURNALS

WAYNE M. HARTWELL

The American Library Association created this last year the Committee on Aid to Libraries in War Areas, headed by John R. Russell, the Librarian of the University of Rochester. The Committee is faced with numerous serious problems and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical periodicals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the coöperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular periodicals to the project should be directed to *Wayne M. Hartwell, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.*

FOURTH SYMPOSIUM ON DEVELOPMENT AND GROWTH

The *Fourth Symposium on Development and Growth* is announced by the Growth Society to be held at North Truro, Cape Cod, Massachusetts from Monday, August 24th to Friday, August 28th. The subject is: *Specific Developmental Responses to Chemical Agents.*

Among the speakers are:

Kurt G. Stern, *Yale Medical School*
Franklin C. McLean, *Chicago University*
E. J. Boell, *Yale University*
Edgar Allan, *Yale Medical School*
Otto A. Bessey, *New York City*
Edward L. Tatum, *Stanford University*
Philip R. White, *Rockefeller Foundation*
A. J. Riker, *Wisconsin University*

The detailed program will be announced later.